SA1. HTS Screen of Novel Drug Libraries for Antiviral Compounds that Block Alphavirus Replication

1. 2015 Primary Screen: *VEEV HTS* identified 940 active samples and 8 out of 12 sent to OHSU had activity in NHDFs. *CHIKV HTS* identified 2,558 active compounds and 5 out of 11 were confirmed.
2. SR screened 347,000 compounds against $VEEV_{TC83}$ using Vero cells and 105 hits were identified. OHSU tested 35 and found 4 actives against CHIKV. SR derived analogs of two compounds (Tetralin-SR-33366 and Quinolone-SR-33394), which have been used for SAR and mode of action studies.
3. In order to both exclude compounds that block virus replication via activation of type I IFN responses and to enhance virus replication, Dr. DeFilippis constructed telomerized human foreskin fibroblast cells that lack IRF3 (THF-ΔIRF3). OHSU validated four anti-VEEV compounds as effective against CHIKV in these cells.
4. Construction and Sequencing of New CHIKV and VEEV Strains: The Alphavirus group has constructed new strains that will facilitate HTS and SAR including a new CHIKV strain expressing nano-Luciferase provided by UNC. Other recent isolates from Puerto Rico have been cloned and sequenced.
5. $VEEV_{TC83}$ has also been modified to encode nluc and is currently being validated at Colorado. $VEEV_{TC83}$-nLuc will be used by SR for SAR studies and the group for antiviral validation studies.

SA2. Validate and characterize antiviral activity and off-target effects

1. The group has developed multiple assays for secondary validation screens and to identify the mode of action for leads. To prevent duplication of effort and maximize experimental efficiency, each individual laboratory of the Alphavirus group has optimized specific assays.
2. **Quinolinones (SR-33394):** SR synthesized >90 analogs. OHSU tested the analogs in virus reduction assays and found the active compounds SR-33394 (EC_{90}=0.77μM), SR-34329 (EC_{90}=0.12μM), SRI-36506 (EC_{90}=4.9μM) and SR-36959 (EC_{90}=0.78μM). SR-34329 is active in VEE replicon assays indicating that the compound targets an early stage in virus replication. Colorado generated a $VEEV_{TC83}$ virus (NSP2 Y101C) that displays resistance to SR-34329. The mutation was reintroduced into the cDNA clone of VEEVTC83 to demonstrate that the single mutation confers resistance to SR-34329 and SR-33394. Antiviral mode of action (MOA) studies are underway for this chemical series.
3. **Tetralins-BenzoAnnulenes (SR-33366):** SR synthesized >125 analogs of SR-33366 for SAR. SR-34963 was found to have about a 10-fold increase in activity against CHIKV with an EC_{90}=0.45μM compared with SR-33366 (EC_{90}=3.2μM). Sequencing of UNC-derived resistance mutants identified changes in the NSP3 macrodomain, which is consistent with MOA studies showing that SR-34963 blocks viral RNA and protein synthesis. SR performed structural biology and modeling analysis and generated a 1.46Å resolution crystal structure of the nsp-3 macrodomain. Additional recent analogs show activity and are under SAR. SR-34963 is broadly active against alphaviruses (ONNV, MAYV, RRV, Una, and VEEV) as well as Flaviviruses (DENV and ZIKV). *In vivo* experiments with analog SR-36498 showed limited activity against CHIKV in mice, and further *in vivo* experiments are underway.
4. **VEEV 2015 HTS:** OHSU confirmed 8 of 12 active hits including: SR-36415 (IC_{90}=0.77μM), SR-36416 (IC_{90}=0.35μM), SR-36420 (IC_{90}=0.13μM), SR-36421 (IC_{90}=0.11μM), SR-36423 (IC_{90}=0.22μM), SR-36424 (IC_{90}=0.06μM), SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM). SR-36426 and 27 were chosen for further SAR. Both work in $IRF3^{-/-}$ fibroblasts indicating that they do not function through IFN. SR-36426 is active against 5 different Alphaviruses and blocks infection prior to viral RNA synthesis. SR-36427 is active against VEEV and Mayaro virus and blocks infection after RNA synthesis. SR generated >50 SR-36427 analogs but none were shown to improve activity profile. Therefore, SR-36427 has been put on hold and a manuscript is in preparation describing antiviral activity for this chemical series. SAR for SR-36426 has shown promising results.
5. **CHIKV 2015 HTS:** OHSU confirmed 5 of 11 hits including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), SR-36767 (IC_{90}=0.09μM), and SR-36768 (IC_{90}=0.23μM). Two compounds (SR-33001 and -36768) were active against 5 different Alphaviruses and SR-36767 was active against 4 Alphaviruses. SR-36767 blocks infection prior to RNA synthesis and is under assessment for MedChem. SR-33001 blocks viral replication at a step after viral RNA synthesis and >25 analogs have been synthesized with promising SAR results.
6. **Project 1, 2, 4 Hits:** DENV compound SR-37014 (IC_{90}=0.4μM) was active against CHIKV. SARS-CoV compounds SR-35742, -35894 and -36565 showed activity against VEEV but not CHIKV.

SA3. Chemical optimization and determination of *in vivo* efficacy of lead compounds

The group has developed a number of models to test *in vivo* efficacy of lead compounds. These include models of: 1) Acute CHIKV infection and joint disease and generation of a mouse-adapted CHIKV strain with enhanced replication and disease; 2) Intranasal inoculation of VEEV; 3) Chronic CHIKV infection and joint disease; 4) Lethal CHIKV and VEEV mouse models; and 5) CHIKV infection of NHP.

Dr. Nicole Haese (Post-Doctoral Fellow-Streblow Lab)
Training Plan

We have designed an individual training plan for Dr. Nicole Haese that will promote her career development by directly addressing strengths and weaknesses in her scientific and academic aptitudes. As a postdoctoral fellow she will have access to the OHSU office for post-doctoral affairs (http://www.ohsu.edu/xd/research/postdocs-students/postdoctoral-fellows-guide/index.cfm). The mission of OHSU's Office of Postdoctoral Affairs (OPA) is to support the career development of all OHSU postdoctoral scholars from arrival to departure. In doing so, the OPA strengthens the research training for all postdoctoral scholars, ensures a consistent and superior postdoctoral experience, and prepares postdoctoral scholars for any professional endeavor they wish to pursue. The most important goal for mentoring students and postdoctoral fellows is to provide them with the skills and experience necessary to be successful at the next level by meeting the following objectives: a) Possess a broad base of established and evolving discipline- and research-specific knowledge and skills; b) Demonstrate effective professional and interpersonal communication skills that allow for interaction with colleagues at all levels; c) Adhere to and reflect professionalism standards and practices within the workplace, institution, and discipline; d) Possess the skills and techniques needed to facilitate effective mentorship, leadership, and management of projects and people; e) Receive training in the responsible conduct of research to improve the ability to make ethical and legal choices; and f) Articulate a plan to pursue a career path of his or her choosing.

Nicole presents her lab work at our formal weekly VGTI meetings, MMI seminars as well as at our combined informal "chalk talk' style lab meetings. Nicole also attends and presents during our Virology/Immunology Journal Club that meets weekly throughout the year. In addition, we have weekly seminars from outside experts and post-docs are given opportunities to meet the speakers and discuss their work. Lastly, Nicole is expected to present her research findings at National and International meetings and attend career development meetings provided by OHSU. The VGTI is an excellent training environment for postdoctoral fellows. The opportunities for learning are immense and the combined experience of the faculty is incredible. The VGTI is an amazing environment that provides a great scaffold for learning how to do 'Team Science'.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	AV-C: A novel small molecule 1-(2-fluorophenyl)- 2-(5-isopropyl-1,3,4-thiadiazol-2-yl)-1,2-dihydrochromeno[2,3-c]pyrrole-3,9-dione capable of blocking Alphavirus replication by activating STING-dependent activity in human cells was characterized and described by Dr. DeFilippis.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: OREGON HEALTH AND SCIENCE UNIVERSITY

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Daniel		Streblow		Project Leader	(b)(4); (b)(6)				13,700.00	4,658.00	18,358.00
2.		Victor		DeFilippis		Co-Investigator					11,801.00	4,131.00	15,932.00
3.		Michael		Axthelm		Co-Investigator					9,255.00	2,314.00	11,569.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **45,859.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			9,616.00	3,654.00	13,270.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
5	3 Resrch Assoc., 1 Microsurgeon, 1 SPF Proj Mgr				79,892.00	18,828.00	98,720.00
6	**Total Number Other Personnel**					**Total Other Personnel**	**111,990.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**157,849.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0969975150000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: OREGON HEALTH AND SCIENCE UNIVERSITY

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		10,000.00
2. Stipends		0.00
3. Travel		0.00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**10,000.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0969975150000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: OREGON HEALTH AND SCIENCE UNIVERSITY

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	35,440.00
2 Publication Costs	0 00
3 Consultant Services	0.00
4 ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Costs: Set-up Fees, Lease Fees and Per Diems	61,342.00
9. Other Expenses	31,569 00
Total Other Direct Costs	**128,351.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**299,200.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. Modified Total Direct Cost	75.0	289,200.00	216,900.00
		Total Indirect Costs	**216,900.00**

Cognizant Federal Agency DHHS, Arif M. Karim, 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**516,100.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Budget_Justification_Yr5_WhitleyU19_OHSU_Streblow_Proj3BCM.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION, YEAR 5

Streblow/DeFilippis (Project 3B)

PERSONNEL:

Daniel Streblow, Ph.D., Co-Investigator, years 1-5: (b)(4) months (b)(4) Dr. Streblow will serve as the OHSU Subcontract PI of Project 3. Dr. Streblow has extensive experience with animal models of infectious disease and has recently teamed up with Dr. Axthelm to develop a non-human primate model of Chikungunya virus infection and disease. His duties will include maintaining active protocols and animal records, coordinating the animal requirements for this Project, facilitating animal experiments, analyzing samples as well as assembling and disseminating data sets produced during this Project. He will ensure timely completion of the proposed work.

Victor DeFilippis, Ph.D., Co-Investigator, years 1-5 (b)(4) months (b)(4) Dr. DeFilippis will be responsible for *in vitro* experimentation, communicating scientific progress, supervision and training of junior staff, overall experimental design and coordination of drug treatment experiments and determination of mode of action of the antiviral compounds. He is also responsible for data analysis, preparation of reports and publications derived from this part of the Project, as well as communication of research results to the scientific community.

Michael Axthelm, Ph.D., Co-Investigator, years 4-5: (b)(4) months, (b)(4) Dr. Axthelm is a veterinary pathologist and an infectious disease specialist with over 20 years of experience investigating mechanisms of viral pathogenesis in non-human primate models, primarily chronic lentivirus and herpesvirus infections. He heads the Infectious Disease Resource that manages the Oregon National Primate Research Center's non-human primate infectious disease protocols. He will advise Al Legasse with respect to coordinating animal selection, protocol implementation, phasing of animal cohorts into the study, and sample and clinical data acquisition. Dr. Axthelm will also advise Mr. Turner in technical aspects of the Project when necessary, including animal sampling procedures, health assessment and anatomic pathology.

Craig Kreklywich, Research Associate, year 5: (b)(4) months, (b)(4) He will be responsible for performing quantitative RT-PCR detection of CHIKV in plasma and tissue samples. He will aid the vet team during necropsy. He is involved in immunohistochemical analysis of CHIKV in tissue samples.

Takeshi Ando, M.D., Microsurgeon, year 5: (b)(4) months, (b)(4) Dr. Ando is a microsurgeon who has been trained in BSL-2 and BSL-3 virological, molecular biological, and animal work. Dr. Ando will be responsible for assisting Dr. Streblow and will be the primary scientist involved in coordinating, conducting, and processing all *in vivo* experiments involving CHIKV.

Michael Denton, Senior Research Assistant, year 5: (b)(4) months, (b)(4) He will be responsible for producing CHIKV titered stocks for infection studies, processing of animal samples, performing titration, CHIKV detection and flow cytometric experiments.

Alfred Legasse, SPF Project Manager, years 4-5: (b)(4) months (b)(4) Mr. Legasse is the Infectious Disease Resource project manager and has over 25 years of experience working with rhesus macaques as a technician, supervisor and project manager. He will be responsible for scheduling and coordinating day-to-day project activities with the Division of Comparative Medicine animal care staff. He will provide quality assurance for staff training and adherence to standard operating procedures developed to maintain personnel and animal safety and the integrity of the study.

Sara Botto, Postdoctoral Scholar, year 5: (b)(4) months, (b)(4) Dr. Botto will work with Dr. DeFilippis in the molecular biological, and animal work. She will be involved in coordinating, conducting, and processing all in vivo experiments.

Rebecca Broeckel, Graduate Research Assistant, year 5: (b)(4) months, (b)(4) Ms. Broeckel will work with Drs. Botto and Defilippis in coordination of drug treatment experiments and general lab protocols for this project.

SUPPLIES (Non-Animal Laboratory)

Antibodies ($2,000/year, years 1-2, $5,558/year, years 3-5)
These are necessary for: 1) Detection of viral replication *in vitro* and for immunohistochemistry; 2) Intracellular cytokine staining assays; 3) Flow cytometry for phenotypic analysis of immune responses to viral infection; and 4) Validation of mode of action studies.

Plasticware/Virus Detection ($47,000/year, years 1-3, $8,500/year, years 4-5)
Disposable plasticware will be required for cell and virus culture, CHIKV titration and virus isolation, and molecular biological work. This includes tissue culture dishes of myriad sizes and layouts, flasks, serological pipettes, disposable pipette tips, microfuge and centrifuge tubes, and disposable screw cap tubes of various sizes for sample storage. This also includes virus detection reagents for quantitative PCR (e.g., Taq polymerase, primers, TaqMan probes, 96-well plates).

Tissue Culture Supplies ($37,200/year, years 1-3, $8,500/year, years 4-5)
These will be required for all cell growth and maintenance as well as virus growth and titration and isolation from tissues. This includes cell culture growth media, animal serum, PBS, trypsin, sucrose, sorbitol, disposable sterilizing filters, antibiotics, and syringes.

Virus Detection Supplies ($20,000/year, years 1-3, $10,000/year, years 4-5)
qRT-PCR will be used for the detection of both CHIKV and DENV. Reagents for virus detection include: Reverse transcription reagents, ABI Master mix containing Taq polymerase, virus-specific primers and TaqMan probes, 96-well optical plates.

LN_2 and CO_2 ($2,882/year, years 3-5)
Liquid nitrogen and carbon dioxide will be needed for incubators, cryopreservation, enzymes for tissue digestion, and tissue fixatives.

ANIMAL COSTS:
Largely fees assessed for animal maintenance (per diem), and fees for surgical services provided by the ONPRC Division of Comparative Medicine staff. The experiments described for years 1-3 were designed to test larger numbers of compounds (or refined compounds) in a mouse model of CHIKV infection and disease. However, in years 4-5 we will test 1-2 of the best candidate compounds in a Rhesus macaque model of CHIKV infection and disease. The prices per year reflect the experimental design.

The number and cost of each of these items are provided in the following other expenses summary:

Estimated number of NHP: Years 4-5, 8 animals.

Rhesus macaque lease fees ($53,902/year, years 4-5 only)
Rhesus macaques cost $6,737.76/animal between the ages of 5-11 years old. The lease fees reflect the portion of the true production costs, and are standardized for all Public Health Service grantees using the ONPRC. Years 4-5: 8 animals x 6,737.76 = $53,902 per year.

Rhesus macaque set-up fees ($1,547/year, years 4-5 only)
$193.41/animal, are charged by the Division of Comparative Medicine to defray the administrative costs of animal selection, records requirements for assignment and initial health assessment to insure healthy animals are assigned to projects. Years 4-5: 8 animals x $183 = $1,547 per year.

Rhesus macaque per diem, ABSL 3 ($5,892/year, years 4-5 only)
$52.61/animal/day for 14 days for 8 monkeys per year in FY4&5.

OTHER EXPENSES:

Necropsy fees ($17,291/year, years 4-5 only)
$2,161.43/animal, 8 animals/year for Years 4 and 5 only (Grade 3 Complex necropsy & histopathology).

Flow cytometry charges ($3,000/year, years 1-5)
We are charged $60/hour of FCM time, which will be used to analyze peripheral blood samples for specific cellular markers as well as when performing intracellular cytokine staining assays for NHPs. We are estimating 50 hours of FCM time per year.

Veterinary time (physical exams) ($3,282/year, years 1-5)
$32.82/animal/hour. We expect roughly 100h total vet time per year. Includes analysis of unexpected complications arising from dugs/infections in mice and NHP.

Slide Preparation and Histology ($3,995/year, years 1-5)
We are charged $5.58 for processing of tissue samples, cutting and mounting, H&E staining. We are estimating that we will need $3,995/year for slide preparation and staining.

Sample collection and drug and agent administration (Surgical Supplies) ($1,000/year, years 1-3; $2,000/year, years 4-5)
Vacutainer blood tubes, needles, syringes and sterile plastic collection tubes and swabs required for obtaining blood samples and tissues from both NHP and mice.

Equipment Maintenance ($2,000/year, years 3-5)
This proposal will require the use of our general laboratory equipment, which must be maintained to properly execute this study. Therefore, we are requesting $3,177 in years 3-5 to maintain the equipment in good working order.

Tuition and Fees ($10,000/year 5)
We request $10,000 in tuition and fees, per OHSU graduate student rates, for Ms. Broeckel.

TRAVEL:

DomesticTravel ($3,000/year, years 1-5)
$3,000/year for Co-Investigators to attend an international meeting pertaining to antiviral therapeutics directed against emerging RNA viruses.

A. COMPONENT COVER PAGE

Project Title: Project 4.1 Identification and characterization of novel drugs that target the Influenza virus polymerase functions

Component Project Lead Information:

Whitley, Richard J.

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of this project is to identify new therapies that target influenza virus replication The global health burden of annual influenza epidemics coupled with the emergence of highly pathogenic strains of influenza virus has highlighted the urgent need for new effective treatments A primary concern with the current drugs (amantadines and neuraminidase inhibitors) used to treat influenza is the development of resistance mutations that negate therapeutic benefit. Published evidence suggests that targeting the influenza virus RNA dependent RNA polymerase (RdRp) is a rational approach for antiviral therapy The RdRp is responsible for a number of functions including 5'cap recognition, endonuclease activity, replication, transcription, and polyadenylation Recently, cryo-EM reconstitution studies identified branched-ribonucleoproteins (RNPs) structures as putative replication intermediates and suggested a mechanism for viral replication by a second polymerase activity on the RNP template [1]. The second polymerase activity is believed to be a function of the polymerase complex Clearly, the RdRp provides multiple functional domains that could be targets for antiviral drug therapy Previous studies showed that mutations in the conserved regions of PB1 subunit of the polymerase complex produce inactive RNA polymerase [2]. We hypothesize that compounds that specifically target the polymerase complex might reduce the frequency of escape mutations, or promote escape mutants that are unfit for replication We have recently identified potential hit compounds from previous HTS screens that significantly inhibit the influenza virus polymerase activity in an RdRp transient assay. These hit compounds were effective against three different strains of influenza viruses in CPE assays. Between Southern Research (SR) and the University of Alabama at Birmingham (UAB), all the necessary primary and secondary assays to perform HTS screening and identify compounds that specifically target the influenza virus polymerase activity have been developed We propose the following specific aims:

Aim#1. Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block influenza virus replication.
Hypothesis and rationale We hypothesize that by targeting the polymerase complex, we might reduce the frequency of mutational evasion because the mutants will be unfit for replication Recent studies demonstrated that the nucleoside inhibitor T-705 induces lethal mutagenesis in H1N1 viruses in vitro resulting in a nonviable phenotype [3]. Targeting the influenza polymerase activity might prove more effective than targeting the viral glycoproteins because there are multiple proteins, as well as protein. protein and protein. RNA interactions, which could be targeted. Our goal is to identify compounds against the conserved regions of influenza virus polymerase subunits that might be effective against multiple viral strains.
Experimental strategy The proposed transient influenza polymerase assay in aim#2 to identify anti-polymerase hits is not adaptable for HTS, and therefore a CPE-based assay will be used as a primary assay to screen novel libraries against influenza viruses. We will screen libraries that have not been previously screened for activity against the viruses covered in this proposal. These libraries are composed of highly diversified small molecules that contain novel and original drug-like features with distinct topologies and diverse functionalities.

Aim#2: Characterize the antiviral activity of hit compounds and identify anti-polymerase inhibitors
Hypothesis and rationale: The existing hit compounds with polymerase inhibitory activity might target one or more subunits of the influenza virus polymerase. The CPE-based HTS screening will identify additional hit compounds that target all stages of the virus life cycle, including multiple functional domains of the influenza RNA polymerase. We have designed an experimental strategy that will focus our analysis on the hit compounds that block post-entry steps of viral infection.
Experimental strategy We will use a variety of secondary assays to identify compounds that specifically inhibit the functions of the viral polymerase complex. Our proposed secondary assays will identify and exclude hit compounds that target viral entry and release, as well as interferon inducers. Following this exclusion process we will examine the remaining positive hit compounds in the transient polymerase assay. Once compound specificity for the viral polymerase is demonstrated, tertiary assays will be performed to determine the target within the polymerase complex

Aim#3: Chemical optimization and determination of the in vivo efficacy of lead compounds.
Hypothesis and Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit the influenza replication complex. Chemical optimization of the effective scaffolds should generate compounds with greater efficacy, selectivity, and bioavailability.
Experimental strategy The hit compounds from the HTS will be triaged and progressed as outlined in the Chemistry core Compounds with the appropriate activity and pharmacokinetic properties will be evaluated using in-house mouse infection models

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded. B2 Project 4.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The initial 892 hits (≥ 50% inhibition in the antiviral assay with cell viability ≥ 80%) identified from the ELVIRA HTS campaign were counter screened in the Mirrorball M2 Immunofluorescence assay (IFA) against H3N2 virus This resulted in 158 compounds that had ≥ 50% IAV inhibition with cell viability ≥ 80%. These compounds were triaged by structural analysis to eliminate those containing undesirable chemical properties which provided 17 compounds In addition, as recommended by the advisory committee, an additional 21,000 SR proprietary compounds were evaluated in H3N2 CPE assay using MDCK cells which resulted in an additional 3 confirmed hits As in other programs, fresh samples, will be acquired, analyzed (purity) and retested in the IFA to confirm activity as well as counter-screened for cytotoxicity effect in the A549 host cells and a CC50 value will be determined The compounds which reconfirm will subsequently be evaluated in the RdRp assay for polymerase inhibition as well as for neuraminidase, hemagglutinin and viral entry inhibition. From these compounds, it is expected that some compounds will be identified which are polymerase inhibitors and potentially compounds that have antiviral activity, but not through any of the known mechanisms. If compounds with this profile are identified, they will be moved into studies to generate resistant mutants, whose genomes will be sequenced to identify the other potential viral targets. From these studies, we will obtain compounds that will move to the Chemistry Core. New compounds that are synthesized will be evaluated in the IFA assay (SAR driving assay) and compounds with EC50 ≤ 20 μM will be tested in the RdRp assay Active compounds will be selected for preliminary structure-activity relationship studies by the Chemistry Core Compounds meeting the established criteria in the IFA and VTR assays will then be tested in primary human small airway epithelial cells using the NanoLuc influenza PATSN (H1N1).

With respect to future in vivo studies, the in vivo team purchased and documented accuracy of a microrectal thermometer for measuring body temperatures of mice as required by IACUC for influenza studies. Lethality studies will be performed to determine the optimal dose for viral intranasal infections in BALB/c mice. Using the NanoLuc reporter IAV, we will perform imaging of infected mice through the UAB Imaging Core to track viral distribution in vivo in the presence or absence of the selected inhibitors. Subsequently, the efficacy and toxicity of lead compounds will be evaluated.

B.2 *What was accomplished under these goals?*

The HTS screening campaign carried out in the third quarter of Year 3, using the ELVIRA cell reporter assay, identified 892 positive hit compounds with confirmed antiviral activity in a dose dependent manner. During the course of this year these compounds were further evaluated for reconfirmation using several independent assays described below.

Virus yield reduction assays

The 892 compounds were evaluated further in a 384-well CPE assay in MDCK cells at concentrations of 10 and 2 μM against A/CA/10/2009 and A/Panama/2001/99 including a cytotoxicity assay with equivalent compound exposure. All data were transferred to the HTS group to upload into the SRI database to identify the compounds that were active against both strains of the virus. Further studies characterized the confirmed hits with a virus yield reduction assay. A total of 180 tests were performed against A/CA/10/2009 (H1N1), A/Panama/2001/99 (H3N2), and/or B/Florida/4/2006. These studies identified 7 confirmed hits with EC_{90} values <20 μM for H1N1 and H3N2 strains.

Immunofluorescence assay using mirrorball technology

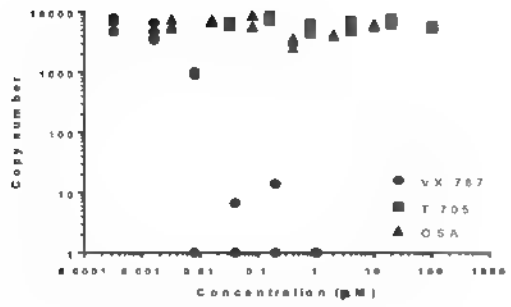
Historically, the CPE assay has resulted in low numbers of confirmed hits and inconsistent results. Therefore, we developed and implemented a new assay for reconfirmation. Thus, an immunofluorescence assay (IFA) using the mirrorball technology and human A549 lung epithelial cells and an antibody directed against the M2 protein of IAV was used to rescreen the 892 compounds found in the primary HTS. From these 892 compounds, 158 compounds showed antiviral activity using IFA with IC_{50} < 50 μM and no cytotoxicity (CC_{50} > 40 μM) on A549 cells. The structures of these compounds are currently being evaluated for further investigation.

Screening of a subset of SR compounds

To identify additional hits, 20,530 compounds from the SR proprietary collection were screened in the MDCK CPE assay. From this set of compounds, 2,155 compounds were tested in a concentration response assay from which six compounds previously known to have anti-viral effects for influenza were identified. An additional 223 active compounds were identified from the remaining 18,275 compounds which were tested at a single concentration. These compounds were retested for a concentration dependent response to confirm activity in the anti-viral CPE assay and were also counter-screened for cytotoxicity against MDCK cells. Thirty-three (33) were confirmed with IC_{50} < 50 μM and a corresponding CC_{50} > 40 μM. These compounds are currently being counter-screened in the ELVIRA reporter assay and the IFA assay to further validate the anti-viral effect.

Development of an RdRp endonuclease assay.

Previous studies with a 384-well assay using NanoLuc influenza strain A/California/04/2009 pdm (H1N1) PATSN in primary human small airway epithelial cells identified a number of compounds with antiviral activity. To further characterize these compounds, a 96-well qPCR assay was developed to assess the inhibition of endonuclease activity of the RdRP complex. The assay specifically detects the formation of chimeric mRNAs that result from the endonucleolytic cleavage of the cellular capped RNAs that are subsequently used to initiate RNA transcription of influenza genes. Specifically, the qPCR assay detects chimeric RNAs that contain the 5' cap and the first 11 base pairs from the U2 snRNA and the influenza PB2 RNA. This assay is unique because it can specifically measure chimeric RNAs formed in the context of antiviral with the native RdRP and native substrates. This assay correctly identified the EC_{50} of the cap binding inhibitor VX-787 and did not detect activity from either T-705 or ribavirin (see graph below). This assay will be further refined and used to identify novel inhibitors of the endonuclease function of the RdRP.


Copy number
10000
1000
100
10
1
VX 787
T 705
OSA
Concentration (µM)

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.	Dr	Richard	J	Whitley		Project Leader	0.00	(b)(4), (b)(6)			24,310.00	7,342.00	31,652.00
2.		Mark		Prichard		Co-Project Leader	0.00				20,822.00	6,288.00	27,110.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **58,762.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
2	Research Supervisor, Research Technician	(b)(4)			27,951.00	9,894.00	37,845.00
2	**Total Number Other Personnel**					**Total Other Personnel**	**37,845.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**96,607.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 063690705
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 063690705

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF ALABAMA AT BIRMINGHAM

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	8,000 00
2. Publication Costs	1,500 00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	5,405.00
7. Alterations and Renovations	0.00
Total Other Direct Costs	**14,905.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**114,512.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	47.0	114,512.00	53,821.00
		Total Indirect Costs	**53,821.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Shon Turner, 214-767-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**168,333.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Whitley Project 4.1 Budget justification Year 5.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Program Director/Principal Investigator (Last, First, Middle): Whitley, Richard J. (Project 4.1)

Budget Justification

Personnel

Richard J. Whitley, MD, PD/PI, (b)(4) months: Dr. Whitley will continue to serve as the UAB Program Director/Principal Investigator for Project 4 investigating the inhibitors of influenza virus. He continues to provide broad oversight of the project and serves as primary liaison with the External Advisory Board, NIH and other external entities, including pharmaceutical companies to determine potential compounds to be developed, and international groups such as the IDSA to determine therapeutic needs.

Mark Prichard, PhD (b)(4) months: Dr. Prichard has conducted research in discovery and development of antiviral drugs for more than twenty years. He continues as the PI of an NIAID contract focused on the evaluation of compounds for antiviral activity against the human herpesviruses and the orthopoxviruses. He continues to work closely with Southern Research on development of assays and evaluation of compounds from the HTS work that has just been completed. He is also looking at sequencing and testing of drug resistance.

Kathy Keith, MS, Laboratory Supervisor (b)(4) months,: Ms. Keith **has** more than 25 years of experience in laboratory work on a number of viruses including HIV, α&β-herpes, influenza, vaccinia and cowpox primarily determining in vitro drug efficacy using different endpoint methods (e.g., ELISA, CPE, plaque reduction, virus yield, hybridization, real time PCR) and under Biosafety levels 2 - 3. She will continue to oversee the antiviral assays and day to day activities for this project in Dr. Prichard's laboratory.

Jessica Eagar, Research Technician (b)(4) months,: Ms. Eagar has more than 5 years of experience in research labs and has increased activity on this project as work has expanded on the assays and in vitro lab work. . She will continue to provide general assistance with day to day operations.

Supplies

Funds are requested for tissue culture, reagents, surgical supplies, PPE, lab ware and miscellaneous laboratory supplies need to conduct the planned compound testing.

Travel

Funds are requested to allow travel of the PD/PI and co-investigators to attend ICAR or other related scientific meetings to present results of the project

Other Expenses

Funds are requested for shipping of materials ($250), publication costs ($1,500), and maintenance of project specific equipment ($1,000). Funding for sequencing core services ($4,405) is also included.

A. COMPONENT COVER PAGE

Project Title: Screening Core - Core B

Component Project Lead Information:

(b)(6), (b)(3) 7 U S C § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overarching goal of the Screening Core (SC) is to identify chemical series with anti-viral effects in high throughput screens against multiple virus targets and to assist in converting them into drugs by providing in vitro biological screening support to the Medicinal Chemistry and Lead Development Core (MCLDC). By screening a unique, common compound collection against each virus, the screening core seeks to identify selective as well as broad-based inhibitors of viral replication in accordance with the theme of the program

Specific Aims

Aim 1: Identify hit compounds for influenza, dengue, Venezuelan equine encephalitis, West Nile, Chikungunya viruses, and SARS Coronavirus. The overall aim of the SC is to identify hit compounds that inhibit replication of influenza (INFV), dengue (DENV), Venezuelan equine encephalitis (VEEV), West Nile (WNV), Chikungunya (CHIKV), and/or SARS CoV. A cytopathic effect (CPE) assay will be employed for screening against VEEV, WNV, CHIKV, DENV and SARS CoV. Different assay readouts will be investigated for screening INFV, including a reporter gene and viral titer assays. The CPE assay for SARS CoV will be run in multiple conditions to identify inhibitors of virus replication by unknown mechanisms of action as well as those specifically targeting CoV fidelity and RNA capping Each of the viruses will be screened using the same 300,000 member library that was selected due to its unique properties with regards to chemical diversity, drug-like properties and potential ability to modulate a variety of biological pathways and targets involved with viral replication By using a common library for all of the assays, compounds that are active across several viruses may be identified and could result in the identification of targets with broad spectrum activity. Primarily this will be accomplished by sharing the compounds among the consortium participants and by establishing an Antiviral Drug Discovery and Development Consortium (AD3C) database using Citrix ShareFile, where all of the assay conditions and screening results will be uploaded. All the Consortium participants have access to this secured site

Aim 2: Perform the assay(s) for each virus to be used to provide the biological support for each virus for structure-activity studies by the Medicinal chemistry and Lead Development Core (MCLDC). As the hits from the HTS assays are developed, a moderate throughput assay is needed for each target to quantify the changes in activity that occurs as structural modifications are made to the hit compounds. These assays (SAR driving assays) will be used in the design-make-test cycle to determine structure-activity relationships that will be important for developing lead series and compounds suitable for in vivo testing As additional mechanistic studies are completed by the various groups, supplemental cell based or biochemical assays may be incorporated into the project.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: B2 Core B.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Aim 1: Completed. No further work planed.

Aim 2: The SC will conduct the anti-viral assays relevant for each project (listed in Table 2 in section B2) to determine both potency (IC50) and efficacy (fold log titer reduction) values for newly synthesized compounds produced by the MCLDC. This work will be an integral component of the iterative design-make-test cycle during the lead generation phase of these projects

B2: Accomplishments under these goals

Aim 1 has been accomplished with the completion of HTS campaigns for all six virus targets (summarized in Table 1). In addition, supplemental funds were awarded in August 2016 (midway through grant year 3) to conduct an HTS campaign for the Zika virus (ZIKV). This work has also been completed using a CPE assay in Vero-CCL 81 host cells and a Zika Paraiba stock obtained from the Diamond lab.

Table 1. Summary of HTS campaigns

Target	Assay	Virus strain	host cell	compounds screened	preliminary hits	validated hits	grant year completed
DENV	CPE	New Guinea (VR-1584)	HEK 293	304,810	2,240	45	Year 1
WNV	CPE	NY99	HEK 293	197,077	2,997	160	Year 3
SARS	CPE	Toronto	Vero E6	305,648	2,492	575	Year 1
CHIKV	CPE	Sri Lanka	Vero E6	197,025	2,558	44	Year 2
VEEV	CPE	TC-83	Vero E6	197,025	940	42	Year 2
FLUV	ELVIRA	H3N2	HEK 293	196,721	3,200	892	Year 3
ZIKV	CPE	Zika Paraiba	Vero-CCL 81	310,438	3,200	1,197	Year 4

Aim 2 is ongoing and so has been partially accomplished. Two SAR driving assays are being used for each virus target to support chemistry efforts. One assay reports the % inhibition of virus effect in the host cell and is used to measure compound potency. The other assay measures the fold log reduction of virus titer in selected wells from the potency assay and is used to measure compound efficacy (summarized in Table 2).

Table 2. Summary of SAR driving assays

Project #	Virus	Anti-viral Potency Assay	Virus Titer Reduction Efficacy Assay	Cell line
1	DENV	IFA	CPE	HEK293
1	WNV	IFA	CPE	TRex 293
1	ZIKV	IFA	CPE	Vero CCL81
2	SARS	VLNLR	VLNLR	Vero E6
3	CHIKV	VLNLR	VLNLR	THF
3	VEEV	CPE	CPE	THF
4	H3N2	CPE	ELVIRA	MDCK/HEK293

Assay Abbreviations

IFA	Immunofluorescence assay (Mirrorball)
VLNLR	Virus linked nanoluc luciferase reporter assay
CPE	Cytopathic Effect assay
ELVIRA	Enzyme linked virus inhibitor reporter assay

Details for each project are described in Core Specific information below.

Core specific information

Project 1. Flaviviruses

Dengue virus

Aim 1 (accomplished Year 1): A CPE assay employing a dengue viral stock prepared in insect cells and HEK293 host cells was used to screen a total of 304,810 compound samples. Using an activity threshold of inhibition ≥ 26.25% (mean + 3xSD of all data), 2,240 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Forty-five (45) compounds were confirmed and validated as hits with IC_{50} < 20 μM and no cytotoxicity. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): Compounds are tested at ten concentrations in an immunofluorescence assay measuring viral protein expression in HEK293 host cell. IC_{50} values are calculated from this data as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

West Nile Virus

Aim 1 (accomplished Year 3): A CPE assay was constructed to identify inhibitors of the viral 2'-O-Methyltransferase. The 2'-O-MTase activity of flaviviruses promotes viral evasion of the Ifit family of genes, a group of host cell IFN-stimulated immune effector proteins. In order to detect inhibitors of virus 2'-O-MTase activity, the HTS assay was performed using transformed HEK 293 cells that expressed Ifit1 when induced by doxycycline. Such compounds will promote the host cell defense mechanism and reduce CPE. The assay also detected compounds that had a direct anti-viral effect since those compounds reduced CPE independently of Ifit expression. A total of 197,077 compounds were screened using HEK cells treated with doxycycline to induce ifit1 expression. Using a statistical threshold of inhibition ≥ 19.03% (mean + 3xSD of all data), 2997 compounds were identified as active. In order to confirm hits and distinguish potential inhibitors of 2'-O-MTase activity from those with direct anti-viral activity, the compounds were retested at 10 concentrations for inhibition of CPE and direct cytotoxicity effects in HEK cells treated with or without doxycycline (i.e. with or without ifit1 expression). IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Hits were deemed confirmed and valid if they had an IC_{50} < 75 μM and no cytotoxic effect. By this criteria, 30 compounds were active only if ifit1 was expressed (i.e. active only in cells treated with doxycycline) and were identified as potential inhibitors of the viral 2'-O-Methyltransferase. An additional 130 compounds were active independent of Ifit expression and identified as those having direct anti-viral effects. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): Compounds are tested at ten concentrations in an immunofluorescence assay measuring viral protein expression in TRex293 host cells. IC_{50} values are calculated from this

data as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Zika Virus (supplemental)

Aim 1 (accomplished year 4): A CPE assay employing a Zika viral stock prepared in insect cells and Vero CCL81 host cells was used to screen a total of 310,438 unique compounds at a single concentration. From this data, 3200 compounds with inhibition ≥ 51.29 % were selected for retesting at 10 concentrations for both anti-viral CPE and direct cytotoxicity effects for which IC_{50} and CC_{50} values were calculated, respectively. A total of 1090 compounds from the SR collection and 107 compounds from the Gilead collection were confirmed and validated showing concentration dependent inhibition of CPE with no direct cytotoxicity. Clustering analysis was performed on 1028 SR hits after eliminating 59 PAINS and 3 duplicate compounds. This resulted in 660 clusters/singletons as follows:

- 12 clusters with 6 or more than 6 members
- 6 clusters with 5 members
- 12 clusters with 4 members
- 50 clusters with 3 members
- 141 clusters with 2 members
- 439 singletons

Supplemental funding was not awarded for follow up chemistry efforts.

Aim 2 (ongoing): In order to determine compound specificity for different flaviviruses, compounds synthesized for the Dengue and West Nile projects are also tested in an immunofluorescence assay measuring Zika viral protein expression in Vero CCL81 host cells. IC_{50} values are calculated from this data as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Project 2. SARS Corona Virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells selected for expression of the SARS CoV receptor (ACE2; angiotensin-converting enzyme 2) were used to screen a total of 305,648 compound samples. Using an activity threshold of inhibition ≥ 80%, 2,492 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects in host cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral CPE and cytotoxicity assays, respectively. Of these, 307 compounds were confirmed and validated as hits showing IC_{50} < 20 μM and SI (IC_{50}/CC_{50}) > 3. An additional 268 compounds were confirmed and validated as hits showing IC_{50} > 20 μM and SI (IC_{50}/CC_{50}) > 3. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer was developed using a recombinant SARS Nanoluc virus produced in the Baric lab. The assay is employed to measure the anti-viral effects of newly synthesized compounds to support development of SAR for hit-to-lead and lead optimization chemistry efforts.

Project 3. Alpha Viruses

Chickungunya virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 50 38% (mean + 3xSD of all data), 2,558 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-four (44) hits were confirmed and validated with IC_{50} < 20 µM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): An assay measuring reporter luminescence as an index of virus titer and replication was developed using a recombinant CHIKV Nanoluc virus produced in the Heise lab. IC_{50} values are calculated from the luminescent signal obtained in virus infected THF cells treated at 10 concentrations of test compound as an index of potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. The nanlouc reporter signal in these wells is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

Venezuelan Equine Encephalitis virus

Aim 1 (accomplished Year 1): A CPE assay employing Vero E6 cells were used to screen a total of 197,025 compound samples. Using an activity threshold of inhibition ≥ 12.12% (mean + 3xSD of all data), 940 samples were identified as active and retested at 10 concentrations for anti-viral CPE and direct cytotoxicity effects using Teleomerized Human Fibroblast (THF) cells. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. Forty-two (42) hits were confirmed and validated with IC_{50} < 20 µM and SI (IC_{50}/CC_{50}) > 10. The list of compounds were submitted to the Core C chemistry team for structural review and analysis to initiate hit-to-lead chemistry.

Aim 2 (ongoing): Compounds are tested at ten concentrations in the CPE assay and IC_{50} values are calculated as an index of compound potency. Media taken from wells treated with 0, 1 and 10 uM test compound are serially diluted over 8 logs and used to infect fresh cells. CPE is measured to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample calculated. The maximal fold reduction in the log of the number of infectious virus particles is used as an index of compound efficacy. These combined data are used to drive SAR for hit-to-lead and lead optimization chemistry efforts.

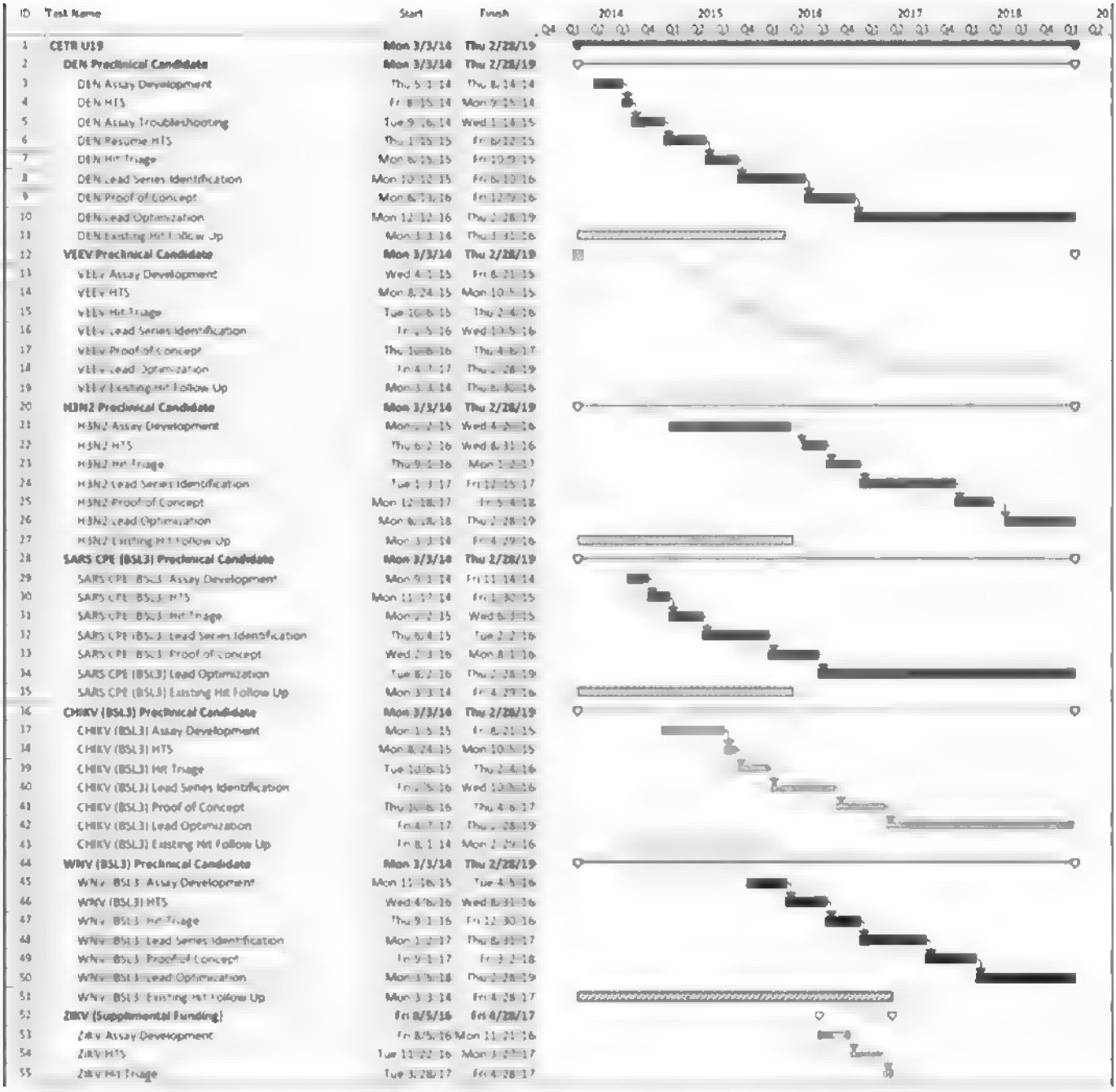
Project 4. Influenza A viruses

Aim 1 (accomplished years 3-4): An enzyme linked virus inhibitor reporter assay was used for HTS. This assay (described in Lutz et al., J. Virol. Methods 2015, 126: 13-20) utilizes an HEK293 cell line engineered to express virus-like negative sense RNA transcripts encoding firefly luciferase flanked by the untranslated regions of influenza A/WSN/33 NP segment. (ELVIRA® Flu A-luc cells). When these cells are infected by influenza A, the virus RdRp transcribes this RNA into mRNA and luciferase protein is produced. Luciferase enzyme activity is then measured as a reporter of virus infection enabling the anti-viral activity of test compounds to be determined by a decrease in luciferase activity. A total of 196,721 unique compounds were screened in HTS using the H3N2 (Udorn) strain. Using an activity threshold of inhibition ≥ 83.45% (mean + 3xSD of all data), 3200 samples were identified as active and retested at 10 concentrations for anti-viral and direct cytotoxicity effects. IC_{50} and CC_{50} values were calculated from the concentration-response data of the anti-viral and cytotoxicity assays, respectively. After eliminating compounds using PAINS filtering, a total of 892 compounds from the SR collection and 185 from the Gilead collection were confirmed and validated showing concentration dependent inhibition of the luciferase reporter signal with no cytotoxicity in ELVIRA® HEK reporter cells. However, when counter screened for CPE in MDCK cells, only 19 compounds showed activity. These results suggest either a high false positive hit rate in the ELVIRA reporter assay or a high false negative reconfirmation rate in the CPE assay. To resolve this issue the 892 hit compounds in the SR collection were retested using an immunofluorescent assay (IFA) measuring M2 protein levels in H3N2 infected A549 cells. Testing each compound at a single concentration in two separate assay runs, 280 compounds were been identified with reproducible activity. These compounds were retested in the IFA assay at 10 concentrations to confirm activity and determine IC_{50} values and counter screened for cytotoxicity effects in the A549 cells. For compounds showing no cytotoxicity on A549 cells as measured by $CC_{50} > 40$ µM the IC_{50} values for the IFA anti-viral effect are as follows: 12 compounds with $IC_{50} < 1$ µM, 48 compounds with 1 µM < $IC_{50} < 10$ µM, and 98 compounds with 10 µM < $IC_{50} < 50$ µM. The structures of these compounds have been sent to the MCLDC for further evaluation. To identify additional hits, 20,530 proprietary compounds in the SR collection were screened in the MDCK CPE assay. Of these 2,155 compounds were tested in concentration response assays from which six compounds previously known to have anti-viral effects for influenza were identified. An additional 223 compounds were identified from the remaining compounds tested at a single concentration. These compounds were retested for concentration response to confirm activity in the anti-viral CPE assay and counterscreened for cytotoxicity against MDCK cells. Of these, thirty-three (33) were confirmed with $IC_{50} < 50$ µM and corresponding $CC_{50} > 40$ µM. These compounds are currently being counter screened in the ELVIRA reporter assay and the IFA assay to further validate the anti-viral effect.

Aim 2 (ongoing): To drive SAR, the immunofluorescent assay measuring surface expression of M2 in H3N2 infected A549 host cells will be used to determine IC_{50} values as an index of compound potency. As with the other projects, media taken from wells treated with 0, 1 and 10 µM test compound will be serially diluted over 8 logs and used to infect fresh ELVIRA cells. The reporter assay will be used to determine the TCID50 of these diluted samples from which the number of infectious virus particles in the original sample will be calculated. The maximal fold

reduction in the log of the number of infectious virus particles will used as an index of compound efficacy.

Timeline of activities



C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

The MCLDC needs data measuring both potency and efficacy of compounds in order to develop SAR for each chemical series Accordingly, the SC has developed and implemented plate-base titer reduction assays for each virus in order to determine the reduction of virus particles (expressed as fold decrease in the log value of virus titer) as a measure of compound efficacy. This data is obtained using media from the assay plates for which virus potency is determined so that the anti-viral effect of a compound is measured both in terms of ability to inhibit virus effects in the host cell as well as reduction in virus titer in the same assay well.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-5069

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6), (b)(3) 7 U S C § 8401				Project Leader	0.00	(b)(4), (b)(6)			33,267.00	14,205.00	47,472.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **47,472.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
11	1 Suprvsr, 4 Biologist, 3 Scientist, 2 Data Sup, 1 Mgr	(b)(4)			129,165 00	55,155 00	184,320.00
11	**Total Number Other Personnel**					**Total Other Personnel**	**184,320.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**231,792.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	2,563.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**2,563.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	85,999.00
2. Publication Costs	0.00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Disposal of HazMat	53,280.00
9. Robot Hours	135,300.00
Total Other Direct Costs	**274,579.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**508,934.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH- Salaries and Benefits	120.0	231,792.00	278,150.00
2. G&A - Total Direct Cost + OH	20.0	787,084.00	157,417.00
3. CFC - Salaries + Benefits	7.3	231,792.00	16,921.00
4. CFC - Total Direct Cost + OH	1.0	787,084.00	787.00
		Total Indirect Costs	**453,275.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number): DHHS, Steven Zuraf, 301-492-4855

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**962,209.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Corrected Year 5 Justification for Screening Core.pdf
(Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Year 5 Budget Justification for the Screening Core

(b)(6), (b)(3) 7 U S C § 8401 Ph.D. will serve as Project Leader of the screening core. (b)(6), (b)(3) 7 U S C § 8401 (b)(6), (b)(3) 7 U S C § 8401 experience in academia and the pharmaceutical industry involving work in over 60 drug discovery programs. (b)(6), (b)(3) has expertise in the development and use of biochemical and cell-based assays in HTS with a focus on translational relevance to ensure that HTS output can be effectively exploited as part of a comprehensive approach to chemical probe and lead generation (b)(6), (b)(3) 7 will have oversight for the automation and execution of the high throughput screens, counter and specificity screens, and biological support for the SAR studies. In collaboration with the PIs and other Co-Investigators (b)(6), (b)(3) will assist in interpretation of the biological results; report, manuscript and patent preparation; and overall project management. (b)(6), (b)(3) will devote (b)(4) (b)(4) months in YR5 to this program.

(b)(6), (b)(3) 7 U S C § 8401 M.S., will supervise and manage the day to day efforts for assay development and screening including scheduling, equipment maintenance and QCing, and data QC. (b)(6), (b)(3) brings her expertise in keeping the Center with an annual operating budget of over $3,000,000 operating efficiently. (b)(6), (b)(3) 7 U S C will devote (b)(4) months in YR5 to the project

(b)(6), (b)(3) 7 U S C § 8401 MS, PMP has five years experience in coordinating and managing research projects. She will work with (b)(6), (b)(3) 7 U S C § 8401 and the other project leaders to ensure a timely and efficient delivery of Core services to the overall program and will devote (b)(4) months in YR5 to the program.

HTS Center Personnel – will be responsible for executing the in vitro biological assays driving SAR including compound handling and informatics support for data analysis:

(b)(6), (b)(3) 7 U S C § 8401 M.S., will be responsible for compound management and drugging for the biological assays. (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 Biologist, B.S., will assis (b)(6), (b)(3) 7 U S C § 8401 in drugging for the HTS. (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 B.S., oversees the HTS informatics group and will be responsible for writing the data templates for the screening effort and data import and analysis and depositing the data with Enterprise Content Management Documentum CenterStage database (b)(6), (b),3) 7 U S C § 8401 manages our ActivityBase software, the Oracle database, and will facilitate transfer of data between the groups including the cheminformatics staff. (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 B.S., will be responsible for importing the data from the plate readers into the analysis software and generating data reports. (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 M.S. will be responsible for the statistical analysis of the high throughput screening data. (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 M.S. will be responsible for running the ZIKV HTS assay and the SAR driving anti-viral assays for all other virus targets. (b)(6), (b)(3) 7 U S C § 8401 has BSL3 certification and is trained to work with select agents. Her efforts will include assay automation and data verification. (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 M.S., will assist in the execution of the SAR driving assays requiring work in the BSL-3 and will be responsible for preparing cells, media, reagents, barcoding plates, and reading plates and the execution of the cell cytotoxicity assays. (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 B.S, will provide laboratory operations support including instrument repair and maintenance (YR5 (b)(4) months)

(b)(6), (b)(3) 7 U S C § 8401 t, will be responsible for growing and maintaining cell lines for supplying the biological assays. (YR5 (b)(4) months)

Other Direct Costs: $83,759 in YR5 has been budgeted for the purchase of biochemical supplies and reagents such as tips, microtiter plates, buffers, media, cells and detection reagents (i.e. Cell Titer Glo). Also, $135,300 in YR5 has been budgeted for the HTS service center charge at a rate of $300/hour for robot usage to prepare compound and assay plates and perform automated screening. This charge includes service contracts, depreciation for the automation equipment, and regular QCing of the equipment. A BSL3 facility charge in the amount of $53,280 is also budgeted in YR4 (listed as HazMat charge). There is a travel request of $2500 for (b)(6), (b)(3) 7 U S C § 8401 to attend the annual CETR meeting.

A. COMPONENT COVER PAGE

Project Title: Medicinal Chemistry and Lead Development Core - Core C

Component Project Lead Information:

Pathak, Asish

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The primary goal of the MCLDC is to provide hit-to-lead analysis, synthetic chemistry, structure-activity relationship (SAR) data and analysis, computational support, and lead optimization chemistry to further the AD3C's mission of developing new broad-based therapeutics for the treatment of infections caused by emerging pathogens. In this role, the MCLDC, in conjunction with the Screening Core (SC; Core B), will be the central focus of the translational research component of the program. As the SC optimizes the novel assays developed by various Research Projects, and subsequently prosecutes the screening campaign, it will be the function of the MCLDC to assess the quality of the hit compounds that emerge, and ultimately to convert novel, tractable hits into potential clinically useful drug candidates with optimized biological and biophysical and drug like properties.
The Specific Aims of Core C, which remain unchanged, are:
Aim 1: Optimize screening hits identified through the primary HTS, dose-response, secondary assays, and counter screens to identify compounds with the activity, selectivity, and pharmacokinetic properties (PK) to warrant animal testing. For example, a typical compound that meets these criteria will have free plasma concentrations in the mouse (or rat, when administered IP, SC or PO) that exceeds the EC50 of the compound's primary activity by 2 to 5 fold for a period of time to be determined by the in vivo model used and the associated in vitro data. The MCLDC will be responsible for all phases of optimization, scale-up, and submission to the SC for testing in the primary SAR screen as well as providing samples to the Center's participants All newly synthesized compounds will be fully characterized using standard spectroscopic and chromatographic tools (HPLC, LC/MS, NMR, MS, and elemental analysis as appropriate) In addition, the MCLDC will be responsible for performing a freedom to operate analysis as well as coordinating the filing of patent applications relating to new compounds when appropriate
Aim 2: Provide integrated informatics support including compound tracking, data capture, data analysis, and data storage, backup, and retrieval. For each assay, an appropriate Protocol ID will be assigned to track data relating to the informatics operations. For each compound synthesized, we will import structures and assign a unique identifier (via our in-house Dotmatics registration database, SRI Numbers) This unique identifier will be used throughout the Center to track compounds and any associated data

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Core C B.2 Accomplishments.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded B.4 Training Core C pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

During Year 5 period of the project, we will continue with our efforts to perform hit-to-lead and lead optimization chemistry approaches on two chemical series (one main and one back-up) in each of the CHIKV and VEEV projects and on one possible chemical series for the WNV and Influenza projects Our aim is to generate at least three hit to lead compounds with reasonable ADME and in vivo PK properties that can be evaluated for potency and efficacy in a mouse model as a proof of concept study These leads will be further optimized with the goal of identifying a pre-clinical candidate(s). We will also pick re-confirmed hits from the recently finished HTS campaign against Influenza virus to start hit to lead and lead optimization medicinal chemistry

B.2. *What was accomplished under these goals?*

Primary goals of Core C include: 1) analysis of high throughput screening data; 2) purchase and re-synthesis of promising hits for potential follow-up chemistry; 3) distribution of these compounds to the various Research Projects for evaluation in relevant assays; 4) medicinal chemistry on selected and confirmed hits (hit-to-lead and lead optimization); 5) Absorption, Distribution, Metabolism, and Excretion (ADME) and PK supports; 6) Structural biology support and; 7) Structure-based virtual screening of commercial libraries against relevant X-ray-derived models.

In continuation with the chemistry activities in Core C from Year 3, Core C performed in all of the above primary responsibilities. Compound Management Group carried out compound repository and all samples were stored in freezers before shipping to various Research Projects locations according to compounds handling and shipping methods. This group is also responsible for maintaining the Dotmatics Compound Management Database provided unique compound identification numbers (SRI Number) as well as used to store biological and ADME data. ADME and Analytical Group carried out *in vitro* ADME analysis and stored data in the Dotmatics system for retrieval and analysis by medicinal chemists. This group also carried out high resolution exact mass (HR-MS) analysis and sample purity by HPLC.

Active compounds from four HTS mass screens [Chikungunya virus (CHIKV), Venezuelan equine encephalitis virus (VEEV), West Nile Virus (WNV) and Influenza virus] which were carried out in Yr. 4, and several closely related analogs were also acquired from various commercial sources for activity re-confirmation and primary SAR- activities. All compounds were analyzed for purity by HPLC and integrity by ^{1}H NMR and HR-MS before registering in to the Dotmatics database to obtain unique identifiers and disperse to various Research Project sites for antiviral activity. Chemical synthesis work in Core C for different projects were prioritized with limited number of bench chemists based on the stages of compound development in hit-to-lead or lead optimization processes as well as availability of different antiviral assays. Medicinal chemistry approaches consist of several steps such as hit(s) identification from HTS data, hits re-confirmation, preliminary ADME properties and visual inspection of structures for uniqueness to prioritize the processes of hit selection and hit-to-lead after which analogs are then synthesized to generate SAR. A lead is then generated to further pursue lead optimization where activity and PK properties are optimized towards the identification of a candidate for evaluation in an animal efficacy model.

Research Project-1 on Flaviviruses include Dengue virus (DENV) and West Nile virus (WNV). Core C continued hit to lead chemistry efforts on three reconfirmed AD3C HTS screen hits (SRI-35847, SRI-33361 and SRI-33376) against DENV from Year 3. For initial hit to lead potentials on all three hits, a total of 195 new analogs were designed and synthesized. These compounds were submitted to Research Project-1 for antiviral activity to evaluate potency and efficacy against VEEV. These compounds were also screened in the Mirror Ball (MB) assay (SAR assay) developed by Core C for antiviral activity. A cell viability assay was also performed to evaluate cytotoxicity and *in vitro* pharmacokinetic properties of active compounds within each series were determined. Some of the Zika HTS hits and actives from other viruses were also tested against DENV virus. Results of these studies are provided in Section G (Core Specific Information).

After the completion of the HTS screening campaign against WNV on 197K+ compounds dose response data of selected compounds was analyzed by Core C from Year 3,. The HTS screens were performed in the following two ways: Screen A) Targeted mechanism: Inhibition 2'-*O*-Methyltransferase A, and Screen B) Secondary mechanism: Direct antiviral effect. Active compounds from both screens were subjected to Pan Assay Interference Compounds (PAINS)

filter followed by clustering analysis. Selected compounds based on their antiviral activity and cytotoxicity data were acquired from commercial sources. The samples were analyzed for their purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting for reconfirmation assay in Research Project-1 sites. These compounds were tested for their antiviral potency (EC_{90}) and efficacy (virus titer reduction assay, VTR). Three re-confirmed compounds from each screen were further pursued as potential hits. Hit optimization studies were carried out on SRI-37776 and SRI-37710. Results of these studies are provided in Section G (Core Specific Information).

Research Project-2 on Coronaviruses includes Severe Acute Respiratory Syndrome (SARS) virus. Hit to lead chemistry was continued from Year 3 on the hit selection from AD3C HTS screen of three compounds (SRI-35293, SRI-33684 and SRI-33911). Newly synthesized compounds were tested in SAR virus NanoLuc (NL) assay developed by Assay Development Core (Core B) as the primary SAR assay. A total of 39 analogs of the first two hits were synthesized and screened for its potency in the NL and cytotoxicity assays to develop preliminary SAR information. The *in vitro* ADME properties of active compounds within each series were also determined. Few potent compounds in SAR assay were tested for their antiviral potency (EC_{90}) and efficacy (VTR) in Research Project-2 laboratory. Fresh samples of three HTS hits (SRI-36096, 36097 and 36100) were also acquired which were not previously available. The samples were analyzed for their purity (HPLC) and integrity (HR-MS and ^{1}HNMR) before submitting for reconfirmation assay in Research Project-2 laboratories. Results of these studies are provided in Section G (Core Specific Information).

Research Project-3 on Alphaviruses includes VEE and CHIK viruses. Core C continued chemistry from Year 3 on a MLPCN re-confirmed hit SRI-33394, which showed excellent antiviral activity in a Normal Human Dermal Fibroblasts (NHDF) cell line against VEEV. Approximately 10 specific analogs were designed, synthesized and submitted to Research Project-3 to test antiviral activity in Year 4. On active compounds from this series, ADME properties, such as human and mouse microsomal stability, aqueous solubility and logD were also determined. Results of these studies are provided in Section G (Core Specific Information). In addition, medicinal chemistry efforts were carried out on two additional reconfirmed HTS hits SRI-36426 and SRI-36427 to generate SAR. The compounds were tested for antiviral potency (EC_{90}) and efficacy (VTR) at HTS using CPE-VTR combo assay followed by reconfirmation assay in Research Project-3 laboratory. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on all active compounds. Results of these studies are provided in Section G (Core Specific Information).

Core C also continued with medicinal chemistry efforts in CHIKV from Year 3. This is one of the most advanced programs among all of the research projects. Medicinal chemistry efforts continued on lead SRI-36498. Lead optimization of SRI-36498 was pursued with a goal of identifying a compound with improved ADME properties such as mouse microsomal stability and solubility (>10 μM) while retaining its antiviral potency and efficacy to test in a mouse model. Approximately 80 new analogs were synthesized and tested for antiviral potency and efficacy using the NHDF (normal human dermal fibroblasts) cell line in Research Project 3 laboratory. Several of the compounds showed excellent antiviral potency and efficacy with reasonable ADME properties. After careful evaluation of activity and ADME profiles of potential lead compounds, SRI-36498 was tested in mouse model for its potency and efficacy at Research Project-3 site. The Structural Biology Group continued its studies towards the target identification of SRI-34963, the parent lead, in the CHIKV virus based on preliminary data generated from virus resistant studies by Research Project 3 labs. Results of these studies are provided in Section G (Core Specific Information).

From the HTS screening campaign against CHIKV, Core C started medicinal chemistry efforts on reconfirmed hit SRI-33001 as a backup series. A total of 47 analogs were designed and

synthesized in Year 4 for SAR studies. New analogs were submitted to HTS to run in NanoLuc-VTR combo assay and to the reconfirmation assay in Research Project-3 laboratory. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability, were evaluated on active compounds. Results of these studies are provided in Section G (Core Specific Information).

In this project there was also an attempt to identify some cross virus active molecules within the alphavirus family and Flaviviruses family. The reconfirmed hits from VEEV and CHIKV hits were cross tested which resulted in several compounds showing activity against each virus. However, none of the compounds showed activity in Flaviviruses family. Results of these studies are provided in Section G (Core Specific Information).

Research Project-4 on Influenza viruses includes three strains (H1N1, H3N2 and H5N1). A fresh solid sample of each of the 19 hits, identified in the third quarter of Year 3 from HTS screen against H1N1 and H3N2 strains using MDCK cells, were evaluated for purity and structural confirmation through HPLC, HR-MS and proton NMR analysis. ADME properties, such as aqueous solubility, log D, and mouse and human microsomal stability were also evaluated on these 19 hits before prioritizing for hit to lead chemistry on re-confirmed hits. Out of 19 compounds submitted, 7 hits were re-confirmed in VTR assay against H1N1 and H3N2 strains using MDCK cells without cytotoxicity. These 7 hits were further evaluated for their polymerase inhibitory effect and 3 hits were found to be active. These 3 hits, which two of them are structurally related, have been selected to follow-up chemistry and a total of 29 analogs of the two structurally related hits have been then synthesized or purchased. However, none of these 29 analogs found to be active when evaluated for their antiviral activity in CPE and VTR assays. Synthesis of analogs of the third hit, which is a known potent CDK inhibitor, was initially planned but due to synthetic challenges of the targeted analogs and the chemical instability, this effort was suspended. The 19 hits were also tested in CPE assay against H3N2 using MDCK cells and none of the tested 19 compounds showed any significant antiviral activity. Later, in Year 4, additional HTS screen on 21K+ SR properitory compounds collection was carried out in CPE assay against H3N2 using MDCK cells. In addition, 890 hits from Year 3 HTS screen were also tested in newly developed Mirror Ball M2 Immunofluroescent assay (IFA) in H3N2 virus using A549 cells. Results of these studies are provided in Section G (Core Specific Information).

B.4 *What opportunities for training and professional development has the project provided?*

We have instituted an undergraduate internship program with University of Alabama at Birmingham Chemistry Department. One undergraduate student worked in Core C and synthesized compounds for the CHIKV program.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

Core C was designed to accommodate hit-to-lead phases in all four projects However, depending on the availability of virus specific assays as well as different stages of success in hit to lead optimization priority changes in the chemistry effort Hence, some projects are much ahead as compare to others. Our most advanced projects are in alphaviruses followed by DENV and WNV. Because of some assay issues in SARS project, medicinal chemistry was slowed down in Year 4 However, now with a fresh plan, the SAR studies will start in Year 5 The HTS data was analyzed from WNV screen, prioritized chemical series of interest in Year 4. Medicinal chemistry on 2 series started in Year 4 which will be continued to optimize lead for proof of concept animal model in Year 5 As, the activities of Core C is dependent of Core B finishing the HTS screens and implementation of SAR assays. The Influenza project HTS hits reconfirmation from reporter assay (ELVIRA) was recently completed using MDCK cells and newly developed M2 protein expression based mirror ball assay. The data is being analyzed. Fresh commercial samples are being ordered and will be analyzed for their purity and integrity As we receive the activity data on fresh samples, we will prioritize which hits to initiate medicinal chemistry Medicinal chemistry efforts in influenza project will soon start in Year 5 We do not anticipate much challenges in Year 5.

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Core-5070

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ashish		Pathak		Project Leader	0.00	(b)(4), (b)(6)			32,168.00	13,736.00	45,904.00
2.		Corinne	E	Szafran		Co-Project Leader	0.00				16,633.00	7,102.00	23,735.00
3.		Mark		Suto		Co-Project Leader	0.00				16,633.00	7,102.00	23,735.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name·

Total Senior/Key Person **93,374.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
3	Post Doctoral Associates	(b)(4)			102,251.00	43,662.00	145,913.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
11	7 Chemist, 2 Scientist, 1 Div. Proj. Mgr. 1 PK Tech				350,031.00	149,463 00	499,494 00
14	**Total Number Other Personnel**					**Total Other Personnel**	**645,407.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**738,781.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	5,125.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**5,125.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	205,000.00
2 Publication Costs	0 00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Analogs	10,250.00
9. PK ADME Supplies	44,801.00
10. Haz Waste	3,331.00
Total Other Direct Costs	**263,382.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**1,007,288.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH-Salaries and Benefits	120.0	738,781.00	886,537.00
2. G&A - OH + Direct Cost	20.0	1,893,825.00	378,765.00
3. FCCM OH	7.3	738,781.00	53,931.00
4. FCCM G&A	1.0	1,893,825 00	1,894 00
	Total Indirect Costs		**1,321,127.00**

Cognizant Federal Agency DHHS Steven Zuraf, 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**2,328,415.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Yr5 Budget Justification - Chemistry Final Core C.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

Budget Justification — Medicinal Chemistry and Lead Development Core

Ashish K. Pathak, Ph.D. (Advanced Research Scientist, Chemistry Department) will serve as a Core Leader of the Medicinal Chemistry and Lead Development Core. He has over 27 years of experience in medicinal and synthetic organic chemistry, including significant experience in anti-infective and vaccine adjuvant drug design. He has established himself as an independent researcher at Southern Research (SR) and previously led research groups in the Department of Chemistry at Western Illinois University as an Assistant Professor before joining SR. Currently, he manages the high-throughput parallel synthesis group and is involved in several internal drug discovery projects as a supervisor in medicinal chemistry. He has extensive experience in all aspects of a medicinal chemistry program, which extends across the spectrum of early lead discovery to lead optimization. He has been PI of two R21 NIH-funded programs in the area of viral vaccine adjuvant discovery. In collaboration with the PIs and other Co-Investigators in the Center, he will oversee all aspects of the medicinal chemistry effort, including hit triage and synthetic target selection, target design, synthesis, and the planning of synthetic routes, compound characterization, structure-activity relationship analysis and interpretation of biological results, cheminformatics and molecular modeling, report, manuscript, and patent application preparation and overall project management of lead optimization chemistry. Dr. Pathak will devote (b)(4) months to the Core during Year 5.

Mark J. Suto, Ph.D. (Vice President, Drug Discovery Division) and Corinne E. Augelli-Szafran, Ph.D. (Director, Chemistry Department) will serve as Co-Core Leaders of the medicinal chemistry core for the proposed project. Each has approximately 30+ years of drug discovery experience across multiple therapeutic areas, including all steps in discovery and development, with a focus on discovering, developing, and advancing new compounds to the clinic in an effective and efficient manner, while ensuring that all of the needed data for regulatory filings are properly gathered and maintained. In addition to experience in early lead identification and discovery, target validation, and lead optimization, Dr. Suto has served on clinical development teams and managed the preparation of drug product for clinical trials. For the current project, Drs. Pathak, Suto, and Augelli-Szafran will work as a team in the selection of hit compounds to move forward, and in the subsequent selection of lead candidates. They will be involved in all aspects of the medicinal chemistry program, including the design of optimal development strategies for individual lead candidates. Dr. Suto will devote (b)(4) months and Dr. Augelli-Szafran will devote (b)(4) months to the Medicinal Chemistry and Lead Development Core in Year 5.

Omar Moukha-Chafiq, Ph.D. (Research Chemist, Chemistry Department) will support Dr. Pathak in running the chemistry core. He has more than 15 years of extensive research experience in the synthesis of potential anticancer, antiviral, antibiotic nucleoside chemistry and small-molecule drug discovery. Because of his broad synthetic background and his experience with solid-, solution-, and liquid- phase methodologies, employing both robotic and manual protocols, he has been the lead chemist on several NIH-funded grant projects. In Grant Year 5, Dr. Moukha-Chafiq will devote (b)(4) months of his time on this project. Mr. Sam Tanner, M.S. and Mr, Larry Bratton, MS; Chemists in Dr. Omar Moukha-Chafiq's group, each will also devote (b)(4) calendar months in Year 5 performing hit to lead chemistry as well as in large scale synthesis.

Mousheng (Mason) Wu, Ph.D. (Structural biologist, Chemistry Department) has extensive training and experience in protein sciences and structural biology including protein expression, protein purification, functional characterization, protein identification and protein structure determination. He holds a Masters of Science degree in Biochemistry and a Ph.D. degree in X-ray crystallography. His lab currently focuses on structure-based drug discovery by providing the details of protein-compound atomic structure to develop new compounds. For this project, Dr. Wu will provide variable efforts once the viral proteins which the anti-viral compounds are targeting are identified. His laboratory will provide support such as cloning, expression and purification of target proteins, characterization of protein-compound binding, and determination of protein-compound structures. The goal of his laboratory in this project is to determine the atomic structure of protein-compound complexes and provide the details to the chemists to assist with the design of more potent compounds. Dr. Wu will devote his efforts for different projects, beginning wit (b)(4) months in Year 5.

Sixue Zhang, Ph.D. (Post-doctoral Researcher, Chemistry Department) has a broad background in

development and application of computational methods for the modeling and understanding of biological systems. He holds a Ph.D. in computational chemistry, structural biology and computer-aided drug design. For this project, Dr. Zhang will provide computational chemistry support for each hit follow-up and lead optimization project, including cheminformatics, clustering analyses, and hit triage filtering, calculation of molecular properties, structure activity relationship (SAR) analysis and model building (such as pharmacophore and homology as appropriate to the given project), searches for commercial analogs of hit compounds, and virtual screening against selected viral target proteins. He will also assist in the preparation of appropriate reports and manuscripts. Dr. Zhang will devote (b)(4) months in Year 5.

Kaleem Ahmed, Ph.D.; Atefeh Garzan, Ph.D.; Theresa Nguyen, Ph.D. and Shuklendu Karyakarte, Ph.D. (Chemists, Department of Chemistry), will provide medicinal chemistry services. They will be directly responsible for day-to-day synthetic activities in the laboratory. They will perform compound synthesis, compound scale-up, and literature searching, and will assist in target selection, analog design, synthetic methodology, compound characterization, data analyses, cheminformatics and molecular modeling. Each of these chemists has several years of research experience in the design and preparation of several different types of compounds and in the analysis of biological data for structure-activity relationships. Each will devote 100% effort to this project over the duration of the grant award in performing hit to lead chemistry and lead optimization on active compounds/series against various viruses (Projects 1-4) and in the scaleup synthesis of lead molecules for animal studies. In Grant Year 5, all 4 chemists will devote (b)(4) months.

David Poon (Chemist, Supervisor of Compound Management, Chemistry Department) has extensive experience in managing in-house synthesized compounds and commercial libraries. He will provide integrated informatics support, including compound tracking, data capture, and data storage, backup, and retrieval. He is in charge of maintaining our in-house Dotmatics registration database, which is used extensively in this program to assign a unique identifier to each compound synthesized or commercially-acquired. This identifier number is used throughout the Center to track compounds and any associated data. He is also responsible for sample preparation and distribution to different project teams and to Core B. He will devote (b)(4) months to this program in Year 5.

Carrie Evans, M.S., PMP (Divisional Project Manager, Drug Discovery Division), has five years of experience in coordinating and managing research projects in the Drug Discovery Division at SR. She will work with Dr. Suto and the other project leaders to ensure a timely and efficient delivery of Core services to the overall program and will devote (b)(4) months in Year 5 to the program.

Donghui Bao, Ph.D. (Research Scientist, Chemistry Department) is the supervisor of the bioanalytical drug discovery laboratory in the Chemistry Department at SR. He has extensive experience in developing and validating efficient bioanalytical methods for quantitative analysis of novel pharmaceuticals, metabolites, and endogenous compounds for use in clinical and non-clinical research and he has a working knowledge of GLP regulations. He also has expertise in quantitative bioanalytical validation, including solid-phase extraction (SPE), high-performance liquid chromatography (HPLC), and mass spectrometry (MS)/MS development and optimization, operation, maintenance, and calibration of liquid chromatography (LC)-MS/MS instrumentation and Rapid Trace SPE instrumentation, research involving animal models including oral, intravenous, and intramuscular dosing, aseptic cell culture techniques, including the use of cultured and freshly isolated hepatocytes, and the handling and analysis of radioisotopes by Liquid Scintillation and Gamma Counting (^{3}H, ^{14}C, ^{51}Cr, ^{125}I). Dr. Bao will be responsible for overseeing PK/ADME studies in all lead optimization projects. He will devote (b)(4) months to this project. Dr. Robert Deimler, Ph.D. (Associate Research Chemist, Chemistry Department) will assist Dr. Bao in day-to-day activities to carry out experimental analytical work in his lab. Dr. Deimler will also perform high resolution mass spectral studies on all final compounds synthesized in this program. Dr. Deimler will devote (b)(4) months in Year 5 to this project.

One technician (TBD, 4.2 calendar months) for animal work will devote 40 hrs/PK study/compound for pharmacokinetic and toxicological profiling of potential drug candidates emerging from the lead optimization program under the direction of Dr. Bao. $43,700 annually has been allocated to cover PK/ADME supplies and animal costs for these studies.

In Grant Year 5, $10,000 have been allocated for the purchase of commercial analogs of hit compounds in order to generate preliminary SAR data. This estimate is based on at 5 scaffolds for follow-up analoging, and 20 commercial analogs being purchased (10-20 mg quantities) for each scaffold at approximately $100/compound.

Six synthetic chemists has been allocated a reagent and supplies budget of $200,000, an estimate based on our previous years in lead optimization chemistry programs. This budget covers starting materials, specialized reagents, solvents, chromatography supplies, resins and solid-phase synthesis supports, glassware, plastic ware, and other disposables as well as spectroscopy and compound characterization expenses. The chemistry supply cost for synthesis is proposed to be $200,000 for Grant Year 5.

Approximately $3,250 is allocated for hazardous waste disposal for Grant Year 5.

$5,000 annually is allocated for the Core Leader and Co-Core Leaders to attend the required NIAID CETR Program Meeting. Which will support meeting registration, abstract submission fees, round-trip airfare, ground transportation, hotel accommodation, and meals for this event.

A. COMPONENT COVER PAGE

Project Title: Project 1.2 Identification and Development of Anti-Flavivirus Lead Drug Candidates

Component Project Lead Information:

Diamond, Michael S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The flavivirus genus comprises >70 single stranded, positive sense RNA viruses that are associated with significant worldwide morbidity and mortality Flaviviruses, which are primarily insect-borne, have been found on every inhabited continent (7) Unfortunately, current therapeutic options for treating diseases associated with these viruses are limited. This proposal builds on existing expertise in small molecule screening for DENV and is designed to identify small molecule compounds with the potential to be developed as antiviral agents The initial screen in this proposal will focus on two medically relevant flaviviruses: dengue viruses (DENV) and West Nile virus (WNV) An existing screening platform will be adapted to screen multiple compound libraries, which include a high representation of nucleoside and nucleotide analogs, potentially compounds that have activity against multiple flaviviruses If broad-spectrum leads with efficacy against multiple viruses can be identified, their further development will be emphasized. In order to enrich for potentially broadly acting compounds, we will focus on compounds that target one of the following important enzymatic activities of the flavivirus NS5 protein' the RNA-dependent RNA polymerase (RdRp), which is essential for replication of the viral RNA genome and the 2'-O-methyltransferase (2'-O-MTase), which is required for the virus to evade the host innate immune response These activities are conserved among the flaviviruses, and similar activities are found in other virus families as well. The overall CETR proposal contains several projects focused on various virus families that are linked by a central screening facility and compound libraries. Therefore, the parallel screening strategies will maximize the likelihood of identifying broad-spectrum antiviral agents that may function across multiple virus families. The specific aims of Project 1 are:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds identify novel inhibitors of flavivirus replication.

Rationale The Southern Research Screening Core (SR SC) has developed and validated cell-based, high-throughput assays for inhibitors of DENV and WNV induced cytotoxicity Initial use of this, or similar, assays has already identified several compounds with antiviral activity. Therefore, this assay will be used to screen novel libraries that have not previously been extensively screened against human pathogens.
Experimental strategy: A CPE based assay will be used as a primary screen for compounds with anti-DENV or anti-WNV activity. Additionally, the WNV screen will be modified in order to allow the detection of compounds that inhibit the viral 2 -O-MTase, thereby sensitizing the virus to the actions of interferon and its effectors. Following the initial screen, "hits" will be evaluated in dose response and cytotoxicity assays in order to determine EC50, CC50, and selective indexes.

Aim 2: Characterize the antiviral activity of hit compounds

Rationale Hit compounds will be further characterized with regard to efficacy and mechanism of action The primary screen will potentially identify compounds that inhibit any of the stages of the viral replication cycle, therefore, secondary experiments are designed to elucidate the stage at which individual compounds act. Additionally, we will also characterize the compounds with regard to breadth of activity against other viruses, and examine the potential for evolution of compound-resistant mutants.
Experimental strategy: We will initially test compounds against sub-genomic viral replicons, which will identify compounds that do not function through affecting viral entry or egress, allowing us to focus on inhibitors of translation, protein processing, or RNA replication We will also identify compounds that function through inhibition of the 2'O MTase, as well as compounds that act non-specifically through induction of interferon or other innate pathways. Compounds will also be evaluated in viral growth assays in order to evaluate the their effect on inhibition of production of infectious progeny virus. Additionally, we will analyze compound effects against multiple viruses and in multiple cell types. Finally, we will test the ability of the virus to develop resistance to individual compounds, as well as characterize any such mutants.

Aim 3: Chemical optimization and in vivo efficacy of lead compounds in animal models of West Nile and Dengue infection.

Rationale: Hit compounds identified and characterized above will be optimized to increase efficacy, selectivity, and bioavailability. These compounds will progress to testing in mouse models of infection.
Experimental strategy Specific compounds and scaffolds will be triaged by the Medicinal Chemistry and Lead Development Core (MCLDC) Compounds with appropriate pharmacokinetic properties will be tested for prophylactic and therapeutic effects in mouse models of WNV and DENV infection.

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded: Project 1.2 B2 Progress on CETR project.2017 Wash U Diamond.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded. B4 Project 1.2.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

With the completion of a HTS by Core B to identify small molecule lead candidate hits that specifically target 2'-O methyltransferase activity, the Diamond laboratory will continue to validate hits and SAR derivatives in the context of expression of mouse and human IFIT1 (the latter with co-expressed IFIT3) We will also work to develop a direct biochemical assay for inhibition of the viral 2'-O methyltransferase in vitro Finally, we hope to initiate in vivo protection studies to assess the antiviral effects of these compounds against WNV pathogenesis.

B.2. Progress on CETR project.

A. Rationale. 2'-O-MTase as a target for small molecule screens. Type I interferon (IFN) cell-intrinsic antiviral defenses protect against many virus infections by signaling host blockade of viral translation, transcription, and replication, thus limiting spread and pathogenesis. Cellular mRNA of higher eukaryotes and many viral RNA are methylated at the N-7 and 2'-O positions of the 5' guanosine cap by specific nuclear and cytoplasmic MTases, respectively. Whereas N-7 methylation is essential for RNA translation and stability, the function of 2'-O methylation and its role in virus infection remained uncertain since its discovery 35 years ago until recently. Studies by members of our group have shown that 2'-O MTase activity of flaviviruses, coronaviruses, and poxviruses promotes viral evasion of Ifit family of genes, a group of IFN-stimulated innate immune effector proteins. Viruses lacking 2'-*O* MTase activity were attenuated in wild type primary cells and immunocompetent animals but were rescued in cells and mice lacking *Ifit1* gene expression. This data is consistent with a model in which 2'-O methylation of the 5' cap of viral RNA subverts innate host antiviral responses through escape of IFIT-mediated suppression, and suggest an evolutionary explanation for 2'-O methylation of cellular mRNA: to distinguish self from non-self RNA. The fact that cytoplasmic viruses cannot use nuclear host 2'O MTases and therefore encode their own viral 2'-O MTases attests to their evolutionary success against their hosts. Nonetheless, given that host 2'-O methylation of cellular mRNA largely occurs in the nucleus, pharmacological strategies that specifically disrupt cytoplasmic viral 2'-O MTase activity could represent a novel class of broad-spectrum antiviral therapy against a number of globally relevant human pathogenic viruses that replicate exclusively in the cytoplasm, including flaviviruses.

B. Goal. Identification of compounds that inhibit viral 2'-O MTase activity and sensitize flaviviruses to the antiviral effects of Ifit1. Compounds that inhibit WNV infection in Ifit1-expressing cells will be tested across a full dose-range for their activity in T-antigen transformed MEFs that ectopically express Ifit1. Small molecules that specifically block 2'-O MTase activity should have little or no inhibitory effect in *Ifit1*$^{-/-}$ cells but should function specifically in isogenic cells expressing Ifit1. Compounds that show this dependence on Ifit gene expression for inhibition of viral replication will be further analyzed as potential inhibitors of viral 2'-O MTase activity. Again, these 'hits' should have no effect on WNV-NS5-E218A, which already lacks 2'-O MTase activity. As final proof of their mechanism of action, lead compounds that sensitize flaviviruses to the effects of Ifit1 in cell culture will be tested *in vivo* for their ability to differentially inhibit flaviviruses in *Ifit1*$^{+/+}$ and *Ifit1*$^{-/-}$ mice.

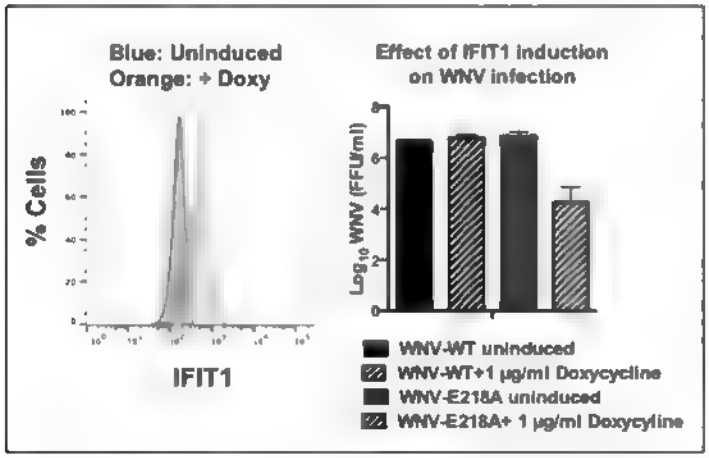


Figure 1. Induction of Ifit1 in 293T cells with doxycycline. Left. Flow cytometry histograms. Right. Viral yield assay at 48 h

C. Progress. Generation of inducible Ifit1-expressing 293T cells.

1. Production of doxycycline-inducible 293T cell line expressing mouse Ifit1. We have generated a 293T cells that expresses Ifit1 under a doxycycline inducible promoter. At baseline, Ifit1 is not expressed but with the addition of 1 µg/ml of doxycycline, Ifit1 is expressed as judged by flow cytometry (**Fig 1, left**). This level of Ifit1 expression is sufficient to inhibit infection of WNV strains lacking 2'-O methylation (WNV-NS5-E218A) but does not inhibit wild-type parent viruses (**Fig 1, right**). These cells were shipped to Southern Research for high throughput screening.

2. Validation of hits from primary screen. A high-throughput screen was performed at SR with untreated (no expression of Ifit1) and doxycycline (+ expression of Ifit1) treated 293T cells to identify compounds that selectively inhibit WNV-WT infection only when Ifit1 was induced. These compounds were shipped to the Diamond laboratory for validation. We confirmed the activity of three of the compounds, one of which is shown (**Fig 2**). Current efforts are aimed at validating these compounds against other flaviviruses (e.g., ZIKV) and at setting a secondary direct screening assay to confirm effects on 2'-O methylation. In addition, SAR is planned for re-screening of additional 'hits'.

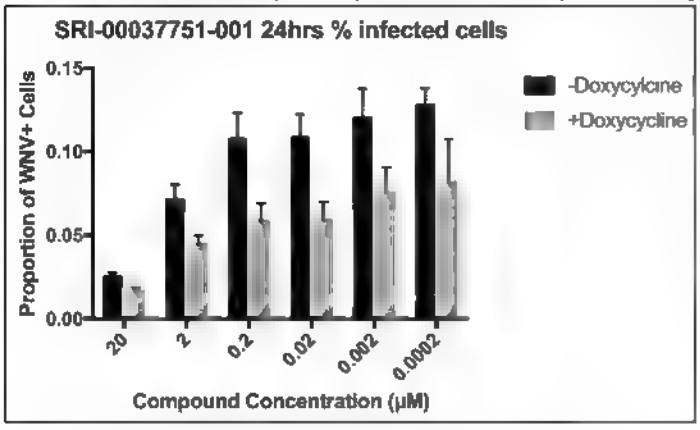


Figure 2. Antiviral effect of SRI compound against WNV-WT only in the doxycycline and Ifit1 expression.

3. Inhibitory activity of human IFIT1 against viruses lacking 2'-O methylaytion. Structural basis for preferential recognition of cap 0 RNA by a human IFIT1-IFIT3 protein complex. Although human IFIT1 inhibits infection of viruses by reducing cap-dependent protein translation, curiously, only mouse Ifit1 more selectively inhibited viruses lacking 2'-O-methylation of their mRNA 5' caps (Daffis et al., 2010; Daugherty et al., 2016). IFIT1 and Ifit1 also may sense and sequester uncapped 5'-ppp viral RNA (Habjan et al., 2013; Pichlmair et al., 2011). We recently determined that human IFIT1 indeed can inhibit viruses lacking 2'-O methylation, but only when the C-terminal region of human IFIT3 is bound. We obtained a crystal structure of cap 0 (m^7GpppN)-RNA-bound human IFIT1 in complex with the C-terminal domain of human IFIT3 ($IFIT3_{CTD}$) that reveals how IFIT-IFIT interactions modulate the binding specificity for RNA ligands. Mass spectrometry and mutational analysis suggest that $IFIT3_{CTD}$ binds to IFIT1 and allosterically regulates the IFIT1 RNA-binding channel and recognition of cap 0 but not cap 1 (m^7GpppNm) or 5'-ppp RNA. In contrast, mouse Ifit3 lacks this key C-terminal domain and does not bind or regulate mouse Ifit1. The IFIT3 interaction with IFIT1 was functionally important for restricting infection of flaviviruses lacking 2'-O methylation in their RNA cap structures. Our new experiments establish differences in regulation of IFIT1 orthologs and define targets for modulating the activity of human IFIT protein function.

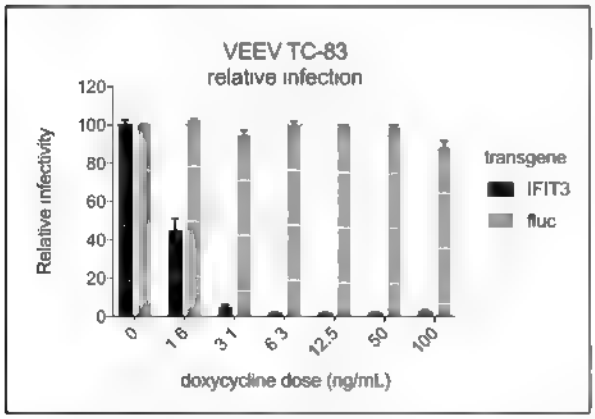


Figure 3. Antiviral effect of human IFIT1 against VEEV only when human IFIT3 is co-expressed.

Indeed, when human IFIT1 is bound to human IFIT3, VEEV, an alphavirus that naturally lacks 2'-O methylation becomes sensitized to the antiviral effects of human IFIT1 (**Fig 3**). Thus, we now can screen our inhibitory compounds for activity in human cells with human IFIT1 to further validate their efficacy.

B.4. Training Opportunities.

The Office of Postdoctoral Affairs (OPA) encourages all postdocs to complete an individual development plan, and recommends using the tool myIDP hosted by Science Careers. New postdocs are introduced to IDPs and the myIDP tool during orientation and workshops are offered throughout the year. OPA recommends that faculty review Individual Development Plans with postdocs at their annual review. IDPs should be reviewed and updated at least annually.

We recognize that postdocs need both information and opportunities to explore the variety of career outcomes pursued by our alumni. OPA has an Education Coordinator and the University employs a full-time career strategist to provide career and professional development training along with Career Talks for postdocs.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Category	Explanation
Research Material	We have generated the doxycycline inducible 293T cell that expresses human IFIT1 and human IFIT3 (as well a C-terminal deletions). Upon publication, we will deposit these cell lines at BEI Resources (ATCC) for use by the greater scientific community. We also will deposit the structural coordinates for the IFIT1-IFIT3-cap 0 RNA complex.

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: WASHINGTON UNIVERSITY

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr	Michael		Diamond		Co-Investigator	(b)(4) (b)(6)				9,350.00	1,964.00	11,314.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **11,314.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	2 Sr. Scientists, 1 Sr. Research Technician	(b)(4)			56,932 00	16,178 00	73,110.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**73,110.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**84,424.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: WASHINGTON UNIVERSITY

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	3,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**3,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0685522070000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: WASHINGTON UNIVERSITY

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	63,946 00
2 Publication Costs	2,000 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Equipment Service Contracts	2,500.00
9. Machine Shop/Computer Maintenance	2,000.00
10. Glassware/BSL3 Waste Disposal	3,000.00
Total Other Direct Costs	**73,446.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**160,870.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC on Campus	52.5	160,870.00	84,457.00
		Total Indirect Costs	**84,457.00**

Cognizant Federal Agency (Agency Name, POC Name, and POC Phone Number)	DHHS, Division of Cost Allocation, 1301 Young Street, Dallas, TX 214-747-3261

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**245,327.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Budget Justification Whitley Diamond-Year 5.pdf (Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

BUDGET JUSTIFICATION

PERSONNEL:

Michael S. Diamond, M.D., Ph.D., Co-Investigator. Professor of Medicine, Molecular Microbiology, Pathology and Immunology at Washington University School of Medicine. Dr. Diamond will devote (b)(4) months to this project and receive (b)(4) months of salary support. He will be responsible for the design and oversight of all investigations occurring in his laboratory. He will actively participate in the planning and execution of experiments, as well as in the generation of progress reports and manuscripts.

James White, Ph.D. Staff Scientist. Dr. White is a Staff Scientists that has been in the Diamond lab for more than 5 years. He has significant experience in flavivirus biology and will perform studies in cell culture to test the effects of lead hits on WNV and other viruses. He will devote (b)(4) months of time to this project and derive (b)(4) months salary from it. He will be responsible for interpreting data and troubleshooting technical problems in consultation with the principal investigator.

Jennifer Govero, PhD. Sr. Research Associate. Dr. Govero has significant experience (~8 years post-Ph.D.) in virology, immunology, and animal models of disease. She will devote (b)(4) months of her time to this project and derive (b)(4) months of her salary accordingly, beginning in FY2. One of her primary roles will be participating in the testing of small molecules in mice. She also will work with Dr. Austin on some of the virological studies in characterizing the lead molecules in vitro and in vivo.

Michelle Noll, Animal Technician. This project requires a significant amount of animal work associated with breeding of Ifit1 KO mice, genotyping, and conducting animal experiments. Ms. Noll, our experienced animal technician, will be responsible for animal husbandry under the oversight of Drs. Govero and Diamond. She will devote (b)(4) months to the project and receive (b)(4) months of salary support in FY 3-5.

EQUIPMENT: No new equipment is needed.

SUPPLIES:

Tissue culture ($7,000). With this project, there will be a considerable amount of tissue culture associated with cell-based validation of small molecule hits and target gene identification. The funds requested will be used for media preparation, growth additives, primary cell culture, serum, antibiotics, plasticware (disposable pipets, pipetman tips, flasks, tubes, cryogenic vials, filtration flasks, sterile bottles).

Molecular Biology Reagents ($3,500). This amount has been budgeted for reagents for molecular cloning (restriction enzymes), proteases, transfection (liposomes, electroporation cuvettes), plasmid DNA and RNA purification kits, electroporation, vectors, bacterial culture supplies, and DNA/RNA/protein electrophoresis. .

Chemicals ($850). This amount has been budgeted for general chemical supplies including buffers, salts, organic solvents, acids, bases, and detergents.

Immunochemical and Reagents ($3,500). This has been budgeted for the direct labeling of antibodies with different fluorophores, for secondary reagents for ELISA, and for intracellular immunofluorescence studies. Some validation screening in different cell types will be performed using immunofluorescence as the readout. This budget also includes time on a shared confocal microscope (~$100/hour).

Real-time RT-PCR ($5,000). We have developed a sensitive and reproducible quantitative real-time RT-PCR for assessing flavivirus replication using an ABI 7000 Sequence Detection instrument. This assay has become our standard for RNA quantitation and will be used to measure viral RNA levels in serum and in tissue and cells. RT-PCR reagents, primers, Taq-Man probes cost approximately $1 per well. In addition, for most samples, a ribosomal or actin RNA control is run for normalization purposes. Also, some of the in vivo studies will use RNA-based methods for quantitation.

Flow Cytometry Reagents and equipment time ($2,500). This amount is for reagents associated with running of the flow cytometer including calibration, buffers, controls, and software licenses. The flow cytometry will be used for screening and validation small molecules that restruct WNV infection.

DNA Oligonucleotides ($1,000). We will use the supplier of the core facility at Washington University which charges approximately $0.20 per base. These oligonucleotides will be used for cloning and sequencing.

Liquid N2/CO2 ($1,000). This amount is budgeted for liquid N2/CO2 bottled gas and related tank rental fees.

Animal pharmacy/sentinal testing/dissection ($1,000 – *FY3-5*). This reflects the costs of anaesthetics and surgical tools for necropsy, and the charges for sentinel testing.

Animal Puchases, Breeding, and Housing ($37,596 – FY3-5). Beginning in FY3, the costs reflect the purchase and breeding for all prophylaxis and therapeutic experiments with small molecules. Also built into these costs are the purchase of Alzet osmotic pumps for drug delivery.

OTHER EXPENSES:

Equipment Service Contracts ($2,500). This money is budgeted for service contracts on all essential equipment to be used with this grant ELISPOT reader (for viral focus forming assays), our 96-well plate flow cytometer, and ABI 7500 TaqMan machine.

Other ($5,000). This money is budgeted for additional costs associated with operation (disposable gowns, gloves, boots, barrier tips) and usage of the BSL3 facilities along with waste disposal (3,000). Funds are also budgeted for machine shop and computer maintenance ($2,500).

Publications ($2,000) we request support on publishing at least one manuscript per year ($2,000).

Travel ($3,000) Travel has been budgeted for one reverse site visit with NIH, one meeting with the Whitley/Nelson laboratories, and a scientific meeting to present data.

A. COMPONENT COVER PAGE

Project Title: Project 2.2 Inhibitors of Coronavirus Fidelity and Cap Methylation as Broadly Applicable Therapeutics

Component Project Lead Information:

Baric, Ralph S

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

Aim 1. To identify and develop inhibitors of CoV high-fidelity replication.

Aim 2. To identify and develop inhibitors of CoV RNA capping activity.

Aim 3. To chemically optimize and test the in vivo efficacy of CoV fidelity and RNA capping inhibitors

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded Project 2 B2.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded Project 2 B4.pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

Plans for the next reporting period are based on continuation of experiments described above Briefly, SR leads will be assessed for activity against MHV, other human CoVs, and pre-emergent bat CoVs We will proceed to probe mechanisms of action by passage for resistance and identification of target and mutations (VUMC). Promising results will trigger an evaluation of in vivo efficacy using murine models of CoV pathogenesis (UNC). We will initiate studies of combinatorial activity profiles and interactions of remdesivir, SR anti-CoV lead compounds, and EIDD-1931. These efforts are designed to enhance activity of, prevent resistance to, and broaden the spectrum of anti-CoV pipeline drugs identified during the preceding four years of U19 support and are distinct from any follow on funding obtained.

B.2. What was accomplished under these goals?

B.2.a. Major activities, specific objectives, significant results, and key outcomes. Our collaborations with Gilead Sciences (GS) and Southern Research (SR) have been productive in preclinical development of candidate therapeutics against SARS-CoV, MERS-CoV and potentially pre-pandemic zoonotic CoVs. An additional candidate lead has been identified.

Remdesivir (Gilead GS-5734). During Year 4 of funding, we published a suite of data in Science Translational Medicine demonstrating that the nucleotide prodrug, remdesivir (GS-5734), can inhibit multiple genetically distinct human and zoonotic CoV (Sheahan et al. 2017. PMC5567817). Importantly, replication of SARS-CoV and MERS-CoV was inhibited in primary human airway epithelial (HAE) cell cultures with submicromolar EC_{50} values. Replication of bat CoVs, prepandemic bat CoVs, and circulating contemporary human CoV were also inhibited, thus demonstrating broad-spectrum anti-CoV activity. In a mouse model of SARS-CoV pathogenesis, both prophylactic and therapeutic administration of remdesivir significantly improved outcomes, reduced lung viral load and improved respiratory function. Recently, the Baric laboratory has demonstrated that both prophylactic and therapeutic remdesivir significantly improved outcomes in MERS-CoV infected mice with improved pulmonary function and reduced viral loads. These data are key for moving remdesivir towards licensure and provide substantive evidence that remdesivir may prove effective against human MERS-CoV infection, circulating human CoV and emerging CoV of the future.

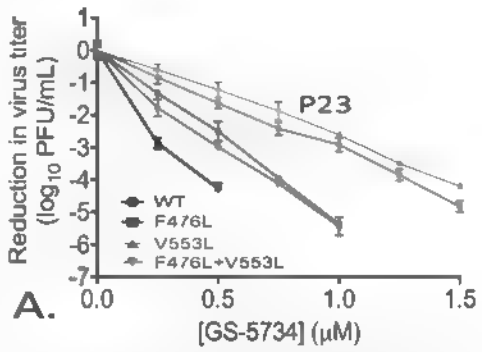


Fig. 1. Mutations in the MHV nsp12-RdRp (F476L and V553L), selected by GS-5734 passage, decrease virus sensitivity to GS-5734 (**A**), but increase sensitivity to cytidine analog EIDD-1931 (**B**). Shown in both is log reduction in titer normalized to 0. P23 (orange) indicates the GS-5734 passage 23 virus. Mutations were identified and introduced in isogenic MHV cloned virus.

To define remdesivir mechanisms of action against CoVs and potential for viral resistance, we passaged the β-CoV murine hepatitis virus (MHV) in the presence of the remdesivir parent nucleoside GS-441524. Two mutations, F476L and V553L, conferring up to 5.6-fold resistance to remdesivir as determined by EC_{50}, were selected in the nsp12-RdRp (**Fig. 1**). These residues are conserved across all CoVs. Resistant viruses were unable to compete with WT in direct co-infection passage in the absence of remdesivir. Introduction of the MHV resistance mutations into SARS-CoV resulted in the same *in vitro* resistance phenotype, and also attenuated SARS-CoV pathogenesis in a mouse model. Finally, we found that an MHV mutant lacking nsp14-exoribonuclease (ExoN) proofreading was significantly more sensitive to remdesivir. Combined, the results indicate that remdesivir interferes with the RdRp even in the setting of ExoN proofreading activity. While partial resistance was achieved with passage in the presence of remdesivir, resistance was attained at the cost of replicative fitness and pathogenic potential. These studies further support development of remdesivir as an antiviral targeting CoV. This work has been submitted to **mBio** (Agostini, Andres, et al. submitted). The work has resulted in this year a published study in Science translational medicine and a submitted report to mBio (under review Dec 2017). In addition two sources of follow-on funding have been identified

Remdesivir (GS-5734). Studies with remdesivir resulted new R01 via the RFA: Partnerships for Countermeasures Against Select Pathogens [RFA-AI-16-034]. The partnership of UNC, VUMC, Gilead, and UTMB will move remdesivir through preclinical development toward IND submission. ***Broad-spectrum antiviral GS-5734 to treat MERS-CoV and related emerging CoV*** (R01AI132178, 8/9/17-8/8/22, P.I. Ralph Baric, Tim Sheahan).

Mechanisms of Resistance of CoVs to nucleoside analogs. Maria Agostini (Vanderbilt) has performed significant studies (paper submitted) of remdesivir activity and resistance. She received F31 support to continue these studies with remdesivir. (b)(6), (b)(3) 7 U S C § 8401

(b)(6), (b)(3) 7 U S C § 8401

SR compounds. Southern Research Institute (SR) compounds SR-36097 and SR-36100 were positive in a SR high-throughput screen (HTS) of >200K small molecules for compounds that inhibited SARS-CoV replication based on CPE reduction. Both hits exhibited activity against SARS-CoV replication in a confirmatory $TCID_{50}$ assay. At a survey concentration of 10 μM, SR-36097 potently diminished SARS-CoV infectious yields in Vero E6 (3 log_{10} PFU titer reduction) and primary HAE cells (2 log_{10} PFU titer reduction) in testing performed at VUMC and UNC, respectively **(Fig. 2).** SR-36100 also potently inhibited SARS-CoV infectious yields in Vero E6 cells (2 log_{10} PFU titer reduction). Neither SR-36097 nor SR-36100 showed activity against MERS-CoV in HAE cultures. Cytotoxicity of SR-36097 and SR-36100 in Vero E6 (ATP levels) and HAE (RNA expression levels of apoptotic signature genes) was insignificant at concentrations ≤10 μM. Taken together, these initial results demonstratate that two SR HTS hits function as potent inhibitors of SARS-CoV replication and warrant further testing for SAR, and testing with other CoVs.

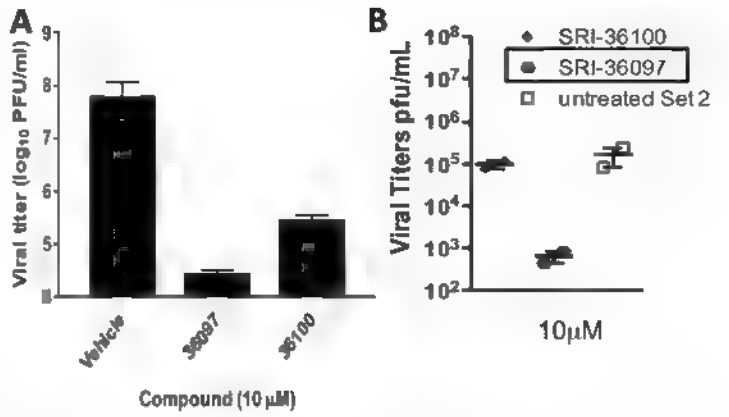


Fig. 2. Effect of SR anti-CoV lead compounds on replication of SARS-CoV in Vero E6 (A) and HAE (B) cells. 36097 demonstrated potent antiviral activity in both culture systems

New Lead Compound. With the surprising success of the nucleoside analog GS-5734 broadly against WT CoVs that encode the proofreading exonuclease, and with the advancement of the GS-5734 in testing and follow on funding, we thought it important to test additional nucleoside analogs that might show broad and potent activity against WT CoVs. EIDD-1931 is a cytidine analogue (Mutation Research. 1980. 72:43) with broad-spectrum antiviral activity and known activity against the alphaviruses Chikungunya and Venezuelan equine encephalitis viruses, as well earlier studies indicating some activity against CoVs but with no development. We requested and obtained the compound from the Emory Institute for Drug Development for testing in our program. We tested EIDD-1931 against group 2a (MHV), 2b (SARS-CoV), and 2c (MERS-CoV) β-CoVs at VUMC and UNC. EIDD-1931 demonstrated profound viral inhibition, with EC_{50} values ranging from 0.004μM to 0.4μM in transformed cells and primary human airway epithelial cultures without demonstrable cytotoxicity, as well as potency with >3 log reduction in virus titer. We performed preliminary studies with EIDD-1931 against remdesivir (GS-5734) resistant mutants. Surprisingly, the mutations resulted in increased viral sensitivity to the unrelated cytidine analog EIDD-1931, suggesting that GS-5734 resistance does not generalize to other nucleoside analogs and that EIDD-1931 may interfere with CoV replication through a different mechanism involving the viral RdRp. They further support the possibility that combinations of different nucleoside analogs might be possible to for synergistic inhibition, limiting emergence of resistance and broadening spectrum.

B.4. Opportunities for Training. Graduate students are active in the project at Vanderbilt and UNC. Individual development plans (IDPs) are generated on an annual basis for all graduate students. These are used for defining key objectives and goals and reviewed on at least an annual basis. For the AD3C program, IDPs include specific goals relevant to the project. IDPs assist in analysis of progress and future training and career development. Construction of the IDP includes creation, review, and updating of biosketches and CVs; these serve as learning tools for presenting professional training and accomplishments in formats relevant to research funding proposals.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RPPR - Project-5072

RESEARCH & RELATED BUDGET - SECTION A & B **FINAL**

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		Ralph		Baric		Project Leader	(b)(4) (b)(6)				18,700.00	4,943.00	23,643.00
2.		(b)(6), (b)(3) 7 U S C § 8401				Co-Investigator					24,472.00	6,874.00	31,346.00
3.		Timothy		Sheahan		Co-Investigator					22,385.00	6,681.00	29,066.00
4.		(b)(6) (b)(3) 7 U S C § 8401				Co-Investigator					21,961.00	6,583.00	28,544.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **112,599.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
2	Post Doctoral Associates	(b)(4)			17,857 00	3,116 00	20,973.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	Research Specialist				18,553.00	5,789.00	24,342.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**45,315.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**157,914.00**

RESEARCH & RELATED Budget (A-B) (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	6,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**6,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	102,036 00
2 Publication Costs	2,000 00
3 Consultant Services	0.00
4. ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Deep Sequencing; Maintenance Contracts	15,000.00
9. Histology, Flow Cytometry	13,000.00
10. Animal per diem, Shipping	8,421.00
Total Other Direct Costs	**140,457.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**304,371.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.0	304,371.00	158,273.00
		Total Indirect Costs	**158,273.00**

Cognizant Federal Agency
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**462,644.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification*	File Name: Baric Year_5_Budget_Justification_Whitley CETR.pdf (Only attach one file.)

RESEARCH & RELATED Budget (F-K) (Funds Requested)

Budget Justification Baric Project Whitley CETR Proposal Year 5

Personnel:

Ralph Baric, Ph.D., Principal Investigator (b)(4) months). Dr. Baric will supervise the overall direction of the animal research agenda of this highly interactive proposal. He will interact closely with Drs. (b)(6) (b)(3) 7 U S C § 8401 and Sheahan, and (b)(6) (b)(3) 7 West to ensure steady progress during the course of the proposal, evaluate results and propose alternative experiments. Dr. Baric will be also be responsible for interacting closely with all research staff, holding regular laboratory meetings, communicating research findings with the (b)(6), (b)(3) 7 U S C § 8401 laboratory, writing progress reports and managing fiscal matters associated with the proposal. Given the extensive interaction and collaboration with Dr. (b) 6), (b)(3) 7 in the past, he will also lead efforts to coordinate and promote research efforts with the groups. Dr. Baric will communicate his findings with Dr. (b) (6 (b) (3) 7 U S C § 8401 on a regular basis via both conference calls and meetings between the two laboratories.

(b)(6) (b)(3) 7 U S C § 8401 (b)(4) months). (b)(6) (b)(3) 7 U S C § 8401 will oversee all select agent research in the facility (b)(6), (b)(3) 7 will test select SARS-CoV and MERS-CoV mutants in primary culture models to evaluate drug candidates in the more advanced human in vitro model. In addition (b)(6), (b)(3) 7 will perform viral passage studies in the presence of drug candidates to identify escape mutations that may arise (b)(6), (b)(3) 7 will report findings regularly to Drs. Baric and (b)(6) (b)(3) 7 U S C § 8401 as well as interfacing with the drug candidate manufacturers.

Timothy Sheahan, Ph.D. Investigator (b)(4) months). Dr. Sheahan has extensive BSL3 experience and has recently rejoined the Baric laboratory as a Research Assistant Professor. He will lead the in vivo drug testing with wild type virus portion of the project in collaboration with Dr. (b)(6) (b)(3) 7 U S C § 8401 as well as performing many of the in vivo experiments. He will be assisted by Drs (b)(6) (b)(3) 7 U S C § 8401

(b)(6) (b)(3) 7 U S C § 8401 (b)(4) months) (b)(6) (b)(3) 7 U S C § 8401 will lead the in vivo testing of compounds with SARS-ExoN I experiments and the in vivo virus evolution experiments proposed in the application (b)(6) (b)(3) 7 in collaboration with Dr. Sheahan will lead our efforts in studying the pathogenesis of SARS-CoV ExoN mutants, derivative ExoN evolved viruses, and conducting in vivo persistent infections/evolution experiments in animals (b)(6) (b)(3) 7 has extensive experience working with ExoN I in mice and is skilled at assembling recombinant SARS-CoV viruses using classic recombinant DNA approaches or synthetic genomic approaches. (b)(6) (b)(3) 7 will work closely with Drs. Baric (b)(6) (b)(3) 7 and Sheahan for experimental design and analysis.

(b)(6) (b)(3) 7 U S C § 8401 (b)(4) months) (b)(6) (b)(3) 7 U S C § 8401 is a new postdoctoral fellow in the Baric laboratory who is learning to work with our infectious clone platform and is interested in virus infection of primary human cell types. (b)(6) (b)(3) 7 U S C § 8401 will work with (b)(6) (b)(3) 7 U S C § 8401 to analyze the drug studies in primary human lung cells and will report all findings to Drs. Baric, Sheahan and (b)(6) (b)(3) 7

(b)(6) (b)(3) 7 U S C § 8401 (b)(4) months). (b)(6) (b)(3) 7 U S C § 8401 is a new postdoctoral fellow in the Baric laboratory who is training now to independently enter the Baric BSL3 laboratory (b)(6), (b)(3) 7 U S C § 8401 has extensive experience infecting mice with human influenza virus and will be working with Drs. Sheahan and (b)(6), (b)(3) 7 U S C § 8401 to perform the animal infections, assay samples and to analyze data. She will communicate all findings to Drs. Baric, Sheahan and (b)(6), (b)(3) 7

(b)(6), (b)(3) 7 U S C § 8401 (b)(4) months (b)(6) (b)(3) 7 U S C § 8401 has extensive BSL3 experience and will assist with viral titration assays and BSL3 animal husbandry. (b)(6) (b)(3) 7 will also be responsible for purchasing supplies, maintaining stocks in the BSL3 and will support Drs. (b) 6) (b)(3) 7 J S C § 8401 and Sheahan's research efforts as needed.

Fringe Benefits: Faculty/Staff: 23.293% Social Security and Retirement; $5,869/FTE Health Insurance. Post-doctoral Research Associates: 8.99% Social Security and benefits; $4,318/FTE Health Insurance.

Travel

Travel: ($6,000): Domestic Travel: Funds are requested for the Project Leader and staff to attend 2 scientific conferences and the annual CETR U19 meeting in Bethesda each year. This allows program faculty and

fellows to communicate results, develop collaborations and share research interests. International Travel: Funds are requested to allow team members to attend the 2018 ICAR meeting in Portugal to present current data and renewal and establish collaborations.

Supplies:

Molecular Biology Reagents ($7,000) Assembling recombinant SARS-CoV and MERS-CoV requires large amounts of highly expensive restriction enzymes (e.g., BsmB1, etc.) and large amounts of DNA ligase. In addition, funds are requested for DNA markers, high quality T7 RNA polymerase, and protein and nucleic acid markers. As sequence confirmation is critical prior to assembly of full length genomic cDNA, funds are also requested to sequence modified genomic fragments following introduction of ExoNI mediated mutations.

Synthetic DNA ($5,500) Funds are requested for the purchase of synthetic DNA fragments which are primarily purchased from small biotec companies like Blue Heron or Bio Basic Inc. at costs of about $0.35/base. Our budget allows for ~40,000-bp of synthetic gene synthesis/yr, sufficient for our needs over the course of this project and will allow for the rapid assembly of recombinant viruses bearing different ExoNI derived mutations.

BL3 Protective Gear ($10,000) Personnel wear powered air purifying HEPA filtered breathing apparatuses, wear tyvek suits, tyvek aprons, hoods, booties and are double gloved when entering the BSL3 facility. These materials are expensive as the HEPA, organic chemical filters and even batteries must be replaced every ~6 months, and the tyvek suits are disposable. Moreover, the PAPR (powered air breathing apparatus) are expensive and must be replaced every ~2 years from normal wear and tear, and daily contact with EPA disinfectants. Personnel use high quantities of disinfectants like ethanol, Clorox and other EPA approved disinfectants in maintaining a safe working environment in the BSL3. Personnel spray down tyvek suits, etc. with alcohol or related disinfectants in the process of deconing and leaving the BSL3 facility. All materials that leave the BSL3 must be disinfected, packaged in disinfected, sealed containers, which are disinfected prior to removal from the BSL3 facility. In addition, funds are requested to help defray costs associated with the decontamination and maintenance of the BSL3 laboratory each year.

Miscellaneous ($6,036) Monies are requested to purchase glassware, pipettes, etc. used in day to day virologic and cell culture procedures as well as in growing, titering and characterizing virus growth in vitro. Funds are also requested to purchase chemicals, reagents, paper products, gloves, micropipetors, autoclave supplies, plastic tips, water baths, and other small equipment items that typically have short half lives in laboratory settings.

Computer Supplies ($1,000) Funds are requested for project specific computer and software upgrades over the course of the proposal.

Tissue culture ($40,000) Funds are requested to purchase mature human airway epithelial cell (HAE) cultures for drug testing assays. Each culture is $100. We anticipate requiring 300 HAE cultures. In addition, we are requesting funds to purchase cell culture supplies and plasticware to perform virus plaque assays and general tissue culture work.

Immunology Reagents ($6,000) Funds to purchase supplies for flow cytometry and ELISA based immune assays are requested to cover the cost of purchasing fluorescently tagged antibodies for the purposes of immune-phenotyping inflammatory cells.

Animals ($26,500) Funds are requested to purchase ~80 each- SCID ($68), RAG ($124), young BalbC ($25), aged BalbC ($20), young B6 ($20) and aged B6 ($18) mice at the indicated prices per mouse. In addition, funds are requested to purchase ~60 golden Syrian hamsters ($43). These monies are essential for evaluating drug efficacy across hosts of differing susceptibilities to lethal infection, and to test drug efficacy in at least two animal species.

Other Expenses:

Animal per diem ($7,800) SCID, RAG and the young BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~30 days prior to the start of experiments (5 animals per cage x 30 days x 0.65 per cage). The aged BalbC/B6 animals will be purchased and housed in UNC animal facilities for ~90 days prior to the start of experiments (5 animals per cage x 90 days x 0.65 per cage).

Deep Sequencing ($10,000) The ExoN mutator phenotype results in high mutation rates which must be accessed by ultra deep sequencing methods like RNAseq, including informatics support to analyze the data. This also includes funds for supplies to generate amplicon library and to prepare the library for sequencing. As such, we anticipate significant sequencing costs over the duration of this proposal.

Maintenance Contracts ($5,000) Several instruments in the Baric Laboratory that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes, BSL3 autoclaves) require service contracts for regular maintenance and repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology ($5,000) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC Chapel Hill. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

Flow Cytometry ($8,000) UNC-Chapel Hill provides a core facility with advanced analytical cytometers that can resolve >6 colors at a time, which is needed when delineating subsets of inflammatory cells following infection.

Publication costs ($2,000) Funds are requested to cover the publication of manuscripts.

Shipping ($621) Funds are requested to cover the costs of shipping samples/viruses to the (b)(6), (b)(3):7 U S C § 8401 laboratory for analysis over the course of the proposal.

A. COMPONENT COVER PAGE

Project Title: Project 3.2 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

(b)(6) (b)(3) 7 L S C § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphavirus species derive evolutionarily from the New World [e g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses] Two distinctive virus-dependent pathologies are manifest during Alphavirus infection Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication This demonstrates that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease Since the target of this class of inhibitors, namely RNA-dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds directed against these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4) In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World) This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique libraries. Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity Initial use of this assay has already identified several compounds with antiviral activity against VEEV Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, "hits" will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50, CC50, and selective indexes.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify 1) Breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) Cell type-specificity (biologically relevant cells), 3) Targets of antiviral compounds, and 4) Ease of developing resistance phenotypes Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds
Rationale Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded Project 3 B 2 Accomplishments pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

1.Quinolinones (SR-33394): This compound series inhibits an early step in the viral lifecycle before or during viral RNA synthesis. We will define the mechanism of action for this class of compounds using in vitro assays. In addition, we will develop assays to assess nsp2 helicase and protease activities in an effort to define SR-33394 mode of action and help drive future HTS efforts We will charactenze the SR-34329 resistant phenotype for VEEVTC83 (nsp2-y101c mutation) for resistance against other analogs in this series. We also will assess the impact of quinolinone resistance on viral fitness in vitro and in vivo. SR plans to synthesize a new series of analogs based upon current SAR, which will be tested for in vitro activity against VEEV and other alphaviruses. Further SAR and testing will be dependent upon the results of the new analog series. We are hoping to be able to test the most active quinolinone analog in vivo during the upcoming year In addition, a manuscript will be submitted describing antiviral activity of this chemical series

2.Tetralins-BenzoAnnulenes (SR-33366): Additional SAR for this chemical series will be completed to optimize activity with stability and bioavailability. We plan to finish mechanism of action (MOA) studies for this compound series utilizing resistance phenotype information, in vitro assays and protein/compound binding. Since this compound series displays activity against a wide range of virus families, we will further characterize this property in order to determine the range of activity. We will continue in vivo pharmacokinetic (PK) analysis to determine formulation, dosing amount, route and timing for efficacy studies in our mouse models of CHIKV infection and disease. Three manuscripts describing this chemical series are in preparation (two describing chemical synthesis strategy and SAR and one describing biological activity for this chemical series), and these will be finished within Q1-2 of the next year. Lastly, we are developing assays to assess macrodomain function (ADP ribosyl hydrolase activity) and utilizing nsp3 macrodomain crystal structure information to guide future screen development (HTS and in silico).

3.VEEV 2015 HTS: Further SAR for SR-36427 will be put on hold for the next year Pending mode of action studies, a manuscript describing the antiviral activity for this chemical series (approximately 50 analogs) is under preparation and should be submitted early in the next year SR-36426 displays high activity and low toxicity profiles and SAR performed during the past year was promising with multiple analogs displaying improved activity and solubility SR will continue to provide the group with additional analogs for this scaffold for SAR Since SR-36426 is broadly active against 5 different Alphaviruses, we will determine the breadth of activity against other viruses. MOA studies should be completed for SR-36426 PK analysis will be performed on the most active compounds If a highly active, soluble SR-36426 analog demonstrates good PK qualities then it will be tested in mice for activity against VEEV and CHIKV.

4 CHIKV 2015 HTS: SR-36767 & SR-33001 are the new leads for CHIKV but both compounds also block a number of other Alphaviruses We will continue to perform MOA and breadth of action studies for these two series SR will continue to synthesize new analogs of SR-33001 in order to optimize compound activity and stability. We hope to perform PK analysis and in vivo testing for these two series during the next year.

5.Project 1, 2, 4 Hits We will continue to test additional compounds that are active against viruses from the other projects in order to identify broadly active compounds

B.2 Accomplished under the goals

SPECIFIC AIMS

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 1), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating *in vitro* replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries. Our goal is the identification of lead molecules for further *in vivo* evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.

Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity. Initial use of this assay has already identified several compounds with antiviral activity against VEEV. Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.

Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the VEEV and CHIKV. Following these initial screens, "hits" will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC_{90}, CC_{90}, and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.

Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to virus-specific efficacy and molecular mechanism of action.

Strategy: We will use a variety of secondary assays to identify: 1) breadth of anti-viral activity (test multiple Alphaviruses); 2) cell type-specificity (biologically relevant cells); 3) targets of antiviral compounds; and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of *in vivo* efficacy of lead compounds.

Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.

Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

Progress towards our goals is outlined for each Specific Aim:

SA1 HTS Screen of Novel Drug Libraries for Antiviral Compounds that Block Alphavirus Replication

1. 2015 Primary Screen: *VEEV HTS* identified 940 active samples and 8 out of 12 sent to OHSU had activity in NHDFs. *CHIKV HTS* identified 2,558 active compounds and 5 out of 11 were confirmed.
2. SR screened 347,000 compounds against $VEEV_{TC83}$ using Vero cells and 105 hits were identified. OHSU tested 35 and found 4 actives against CHIKV. SR derived analogs of two compounds (Tetralin-SR-33366 and Quinolone-SR-33394), which have been used for SAR and mode of action studies.
3. In order to both exclude compounds that block virus replication via activation of type I IFN responses and to enhance virus replication, Dr. DeFilippis constructed telomerized human foreskin fibroblast cells that lack IRF3 (THF-ΔIRF3). OHSU validated four anti-VEEV compounds as effective against CHIKV in these cells.
4. Construction and Sequencing of New CHIKV and VEEV Strains: The Alphavirus group has constructed new strains that will facilitate HTS and SAR including a new CHIKV strain expressing nano-Luciferase provided by UNC. Other recent isolates from Puerto Rico have been cloned and sequenced.
5. $VEEV_{TC83}$ has also been modified to encode nluc and is currently being validated at Colorado. $VEEV_{TC83}$-nLuc will be used by SR for SAR studies and the group for antiviral validation studies.

SA2. Validate and characterize antiviral activity and off-target effects

1. The group has developed multiple assays for secondary validation screens and to identify the mode of action for leads. To prevent duplication of effort and maximize experimental efficiency, each individual laboratory of the Alphavirus group has optimized specific assays.
2. **Quinolinones (SR-33394):** SR synthesized >90 analogs. OHSU tested the analogs in virus reduction assays and found the active compounds SR-33394 (EC_{90}=0.77μM), SR-34329 (EC_{90}=0.12μM), SRI-36506 (EC_{90}=4.9μM) and SR-36959 (EC_{90}=0.78μM). SR-34329 is active in VEE replicon assays indicating that the compound targets an early stage in virus replication. Colorado generated a $VEEV_{TC83}$ virus (NSP2 Y101C) that displays resistance to SR-34329. The mutation was reintroduced into the cDNA clone of VEEVTC83 to demonstrate that the single mutation confers resistance to SR-34329 and SR-33394. Antiviral mode of action (MOA) studies are underway for this chemical series.
3. **Tetralins-BenzoAnnulenes (SR-33366):** SR synthesized >125 analogs of SR-33366 for SAR. SR-34963 was found to have about a 10-fold increase in activity against CHIKV with an EC_{90}=0.45μM compared with SR-33366 (EC_{90}=3.2μM). Sequencing of UNC-derived resistance mutants identified changes in the NSP3 macrodomain, which is consistent with MOA studies showing that SR-34963 blocks viral RNA and protein synthesis. SR performed structural biology and modeling analysis and generated a 1.46Å resolution crystal structure of the nsp-3 macrodomain. Additional recent analogs show activity and are under SAR. SR-34963 is broadly active against alphaviruses (ONNV, MAYV, RRV, Una, and VEEV) as well as Flaviviruses (DENV and ZIKV). *In vivo* experiments with analog SR-36498 showed limited activity against CHIKV in mice, and further *in vivo* experiments are underway.
4. **VEEV 2015 HTS:** OHSU confirmed 8 of 12 active hits including: SR-36415 (IC_{90}=0.77μM), SR-36416 (IC_{90}=0.35μM), SR-36420 (IC_{90}=0.13μM), SR-36421 (IC_{90}=0.11μM), SR-36423 (IC_{90}=0.22μM), SR-36424 (IC_{90}=0.06μM), SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM). SR-36426 and 27 were chosen for further SAR. Both work in $IRF3^{-/-}$ fibroblasts indicating that they do not function through IFN. SR-36426 is active against 5 different Alphaviruses and blocks infection prior to viral RNA synthesis. SR-36427 is active against VEEV and Mayaro virus and blocks infection after RNA synthesis. SR generated >50 SR-36427 analogs but none were shown to improve activity profile. Therefore, SR-36427 has been put on hold and a manuscript is in preparation describing antiviral activity for this chemical series. SAR for SR-36426 has shown promising results.
5. **CHIKV 2015 HTS:** OHSU confirmed 5 of 11 hits including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), SR-36767 (IC_{90}=0.09μM), and SR-36768 (IC_{90}=0.23μM). Two compounds (SR-33001 and -36768) were active against 5 different Alphaviruses and SR-36767 was active against 4 Alphaviruses. SR-36767 blocks infection prior to RNA synthesis and is under assessment for MedChem. SR-33001 blocks viral replication at a step after viral RNA synthesis and >25 analogs have been synthesized with promising SAR results.
6. **Project 1, 2, 4 Hits:** DENV compound SR-37014 (IC_{90}=0.4μM) was active against CHIKV. SARS-CoV compounds SR-35742, -35894 and -36565 showed activity against VEEV but not CHIKV.

SA3. Chemical optimization and determination of *in vivo* efficacy of lead compounds

The group has developed a number of models to test *in vivo* efficacy of lead compounds. These include models of: 1) Acute CHIKV infection and joint disease and generation of a mouse-adapted CHIKV strain with enhanced replication and disease; 2) Intranasal inoculation of VEEV; 3) Chronic CHIKV infection and joint disease; 4) Lethal CHIKV and VEEV mouse models; and 5) CHIKV infection of NHP.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 6081952770000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6), (b)(3) 7 U.S.C. § 8401				Consortium PI	(b)(4), (b)(6)				17,128.00	4,577.00	21,705.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **21,705.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
3	2 Senior Scientists, 1 Research Technician	(b)(4)			52,329.00	17,178.00	69,507.00
3	**Total Number Other Personnel**					**Total Other Personnel**	**69,507.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**91,212.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	4,000.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**4,000.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 6081952770000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF NORTH CAROLINA AT CHAPEL HILL

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	45,288 00
2. Publication Costs	0 00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Histology Core	5,000.00
9. Service Contracts and Maintenance	4,500 00
Total Other Direct Costs	**54,788.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**150,000.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	52.0	150,000.00	78,000.00
		Total Indirect Costs	**78,000.00**

Cognizant Federal Agency DHHS, Darryl Mayes 202-401-2808
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**228,000.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: (b)(6), (b)(3) 7 U S C § 8401 A3DC-UNC Justification 2017.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel

(b)(6) (b (3) 7 U S C § 8401 (b)(4) Months, (b)(4) (b)(6) (b),3)7 U S C § 8401 has expertise in viral pathogenesis and viral immunology, and has worked with models of alphavirus pathogenesis, including CHIKV and VEEV for fifteen years. (b)(6), will be responsible for the overseeing the testing of candidate therapeutics for their ability to protect mice from acute CHIKV induced arthritis or VEE-induced viral encephalitis. (b 6), (b)(3) 7 will work in close collaboration with Drs. DeFilippis and Streblow, as well as the other research project leaders to set priorities for which drugs should be tested within the CHIKV or VEE models and to identify promising candidates that show promising in vivo potential so that those candidates can be taken forward for further optimization.

(b)(6), (b)(3) 7 U S C § 8401 (b)(4) Months (b)(4) (b)(6) (b)(3) 7 J S C § 8401 has several years of experience working with pathogen infected mice under BSL-3 conditions. (b)(6), will work in close coordination with (b (6 (b)(3) 7 U S C § 8401 to test candidate therapies for their ability to protection from CHIKV or VEE-induced disease. (b)(6), will administer therapeutics, perform CHIKV and VEE infections, and will monitor infected animals for disease signs and collect tissues to assess viral loads and virus-induced pathology. (b)(6), (b)(3) 7 also directs the day to day operations of the BSL-3 facility where all work with CHIKV and VEE is conducted and will oversee the proper training and compliance of all individuals working within the BSL-3 facility.

(b)(6), (b)(3) 7 U S C § 8401 (b)(4) Months (b)(4) (b)(6) (b)(3) 7 J S C § 8401 has approximately 10 years of experience working with VEE and CHIKV infected mice under BSL-3 conditions. (b)(6), (b)(3) 7 will be responsible for coordinating in vivo mouse studies and will be involved in the administration of candidate therapies and viral challenge studies. (b)(6), will also assist in collection of data to assess the impact of therapeutics on viral loads, virus-induced disease, and virus-induced pathology within joint (CHIKV) and the central nervous system (VEE).

(b)(6) (b)(3) 7 U S C § 8401 (b)(4) Months, (b)(4) . (b)(6) (b)(3) 7 J S C § 8401 has experience working with alphaviruses and alphavirus molecular clones. (b)(6) will be responsible for testing candidate compounds for antiviral activity against CHIKV and VEE, determining what stage in the viral replication cycle the inhibitory compounds are acting, and determining whether resistance mutants arise against the compounds.

Fringe Benefits: Faculty/Staff: 23.293% Social Security and Retirement; $5,869/FTE Health Insurance.

SUPPLIES

The evaluation of candidate therapies against either CHIKV or VEE requires the assessment of viral loads, evaluation of inflammatory cell infiltration and pathology within these tissues. Therefore funds are requested to cover the costs of tissue culture consumables (plastic ware, media, serum) required for the assessment of viral loads within the joints or CNS. We will also need to generate viral stocks, as well as generate infectious clones containing potential escape mutants and funds are requested to cover the costs of the molecular biology supplies needed for those purposes. We are also requesting funds to cover the cost of purchasing adult C57Bl/6 mice, which will be used for testing candidate therapies against both CHIKV and VEE, as well as funds to cover the cost of supplies needed to house these animals within our BSL-3 laboratory. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective gears, such as gloves, tyvek suits, and PAPRs.

TRAVEL

Funds are requested for the Project Leader, and 2 investigators to attend 1 scientific meeting to present findings and interact with other scientists in the field and to attend programmatic meetings.

OTHER EXPENSES

Equipment service contracts ($4,500): Several instruments in the Heise Laboratory that will be used in these studies (4deg centrifuge, CO2-incubators, microscopes) require service contracts for regular maintenance and

repairs when needed. These are sophisticated instruments, so the repairs require specialists with appropriate tools and particular replacement parts. A fraction of these costs are included here.

Histology Costs ($5,000) Histology slides from paraformaldehyde fixed tissues are prepared on a fee for service basis at UNC. Given the large number of tissues to be analyzed each year, we are requesting funds to cover this tissue/slide preparation and staining costs.

A. COMPONENT COVER PAGE

Project Title: Project 3.3 Novel Therapeutic Strategies Targeting Re-emerging Alphaviruses

Component Project Lead Information:

MORRISON, THOMAS E

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphavirus species derive evolutionarily from the New World [e g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses] Two distinctive virus-dependent pathologies are manifest during Alphavirus infection Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication This demonstrates that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease Since the target of this class of inhibitors, namely RNA-dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds directed against these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 3), Coronaviruses (Project 2), and Influenza (Project 4) In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating in vitro replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World) This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique libraries. Our goal is the identification of lead molecules for further in vivo evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.
Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity Initial use of this assay has already identified several compounds with antiviral activity against VEEV Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.
Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the Alphaviruses VEEV and CHIKV. Following these initial screens, "hits" will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC50, CC50, and selective indexes.

Aim 2: Validate and characterize antiviral activity and off-target effects.
Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to efficacy and mechanism of action.
Strategy: We will use a variety of secondary assays to identify 1) Breadth of anti-Alphavirus activity (test multiple Alphavirus species); 2) Cell type-specificity (biologically relevant cells), 3) Targets of antiviral compounds, and 4) Ease of developing resistance phenotypes Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of in vivo efficacy of lead compounds
Rationale Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.
Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded Project 3 B 2 Accomplishments pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

NOTHING TO REPORT

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

1.Quinolinones (SR-33394): This compound series inhibits an early step in the viral lifecycle before or during viral RNA synthesis. We will define the mechanism of action for this class of compounds using in vitro assays. In addition, we will develop assays to assess nsp2 helicase and protease activities in an effort to define SR-33394 mode of action and help drive future HTS efforts We will charactenze the SR-34329 resistant phenotype for VEEVTC83 (nsp2-y101c mutation) for resistance against other analogs in this series. We also will assess the impact of quinolinone resistance on viral fitness in vitro and in vivo. SR plans to synthesize a new series of analogs based upon current SAR, which will be tested for in vitro activity against VEEV and other alphaviruses. Further SAR and testing will be dependent upon the results of the new analog series. We are hoping to be able to test the most active quinolinone analog in vivo during the upcoming year In addition, a manuscript will be submitted describing antiviral activity of this chemical series

2.Tetralins-BenzoAnnulenes (SR-33366): Additional SAR for this chemical series will be completed to optimize activity with stability and bioavailability. We plan to finish mechanism of action (MOA) studies for this compound series utilizing resistance phenotype information, in vitro assays and protein/compound binding. Since this compound series displays activity against a wide range of virus families, we will further characterize this property in order to determine the range of activity. We will continue in vivo pharmacokinetic (PK) analysis to determine formulation, dosing amount, route and timing for efficacy studies in our mouse models of CHIKV infection and disease. Three manuscripts describing this chemical series are in preparation (two describing chemical synthesis strategy and SAR and one describing biological activity for this chemical series), and these will be finished within Q1-2 of the next year. Lastly, we are developing assays to assess macrodomain function (ADP ribosyl hydrolase activity) and utilizing nsp3 macrodomain crystal structure information to guide future screen development (HTS and in silico).

3.VEEV 2015 HTS: Further SAR for SR-36427 will be put on hold for the next year Pending mode of action studies, a manuscript describing the antiviral activity for this chemical series (approximately 50 analogs) is under preparation and should be submitted early in the next year SR-36426 displays high activity and low toxicity profiles and SAR performed during the past year was promising with multiple analogs displaying improved activity and solubility SR will continue to provide the group with additional analogs for this scaffold for SAR Since SR-36426 is broadly active against 5 different Alphaviruses, we will determine the breadth of activity against other viruses. MOA studies should be completed for SR-36426 PK analysis will be performed on the most active compounds If a highly active, soluble SR-36426 analog demonstrates good PK qualities then it will be tested in mice for activity against VEEV and CHIKV.

4 CHIKV 2015 HTS: SR-36767 & SR-33001 are the new leads for CHIKV but both compounds also block a number of other Alphaviruses We will continue to perform MOA and breadth of action studies for these two series SR will continue to synthesize new analogs of SR-33001 in order to optimize compound activity and stability. We hope to perform PK analysis and in vivo testing for these two series during the next year.

5.Project 1, 2, 4 Hits We will continue to test additional compounds that are active against viruses from the other projects in order to identify broadly active compounds

B.2 Accomplished under the goals

SPECIFIC AIMS

The goal of this project includes identification of novel small molecules capable of inhibiting replication of diverse members of the Alphavirus genus. Alphaviruses are arthropod-transmitted RNA viruses comprising seven antigenic complexes that include multiple Biodefense Category B and C priority pathogens. Alphaviruses are broadly comprised of geographically derived clades: New World [e.g. Eastern (EEEV), Venezuelan (VEEV), and Western Equine Encephalitis (WEEV) viruses) and Old World [e.g. Chikungunya (CHIKV), Ross River (RRV), Semliki Forest (SFV), and Sindbis (SINV) viruses]. Two distinct pathologies are manifest during Alphavirus infection. Neurological disease including encephalitis is primarily associated with New World species and can present high mortality rates especially in hosts with weakened or immature immune systems as well as the young and aged populations. Arthralgia and inflammatory syndromes are typically associated with Old World species and while these are uncommonly fatal they can elicit incapacitating effects that persist long after viral clearance. Importantly, CHIKV is currently undergoing a severe re-emergence in areas around the Indian Ocean and Caribbean, an event that has involved evolutionary adaptation allowing inter-host transmission via mosquito species present in North America. Currently no FDA approved vaccines or antiviral therapeutics are available to prevent Alphavirus infection or treat Alphavirus-associated disease. Importantly, Alphavirus genomes mutate rapidly, greatly facilitating spontaneous changes in their host and vector ranges and virulence, and escape from prior immunity. We have found that the nucleoside analog Ribavirin inhibits CHIKV vRNA synthesis and replication, demonstrating that nucleoside and nucleotide analogs may represent viable therapeutic agents against Alphavirus disease. Since the target of this class of inhibitors, namely RNA- dependent RNA polymerase (RnRp) activity, is well conserved among the Alphaviruses, compounds that impact these enzymes should target multiple species and perhaps other RNA virus clades such as Flaviviruses (Project 1), Coronaviruses (Project 2), and Influenza (Project 4). In light of this, experiments outlined in our proposal will utilize an established Alphavirus screening platform to examine a large, previously unexplored chemical library, heavily occupied by nucleoside and nucleotide analogs, by evaluating *in vitro* replication of two clinically relevant human Alphaviruses namely CHIKV (Old World) and VEEV (New World). This assay has been used to screen a compound library against VEEV and identified >100 that are active against VEEV. Subsequent work will involve validation and mechanistic characterization of these efficacious compounds as well as additional ones identified in our primary HTS using unique molecular libraries. Our goal is the identification of lead molecules for further *in vivo* evaluation using both murine and nonhuman primate models of infection. Parallel screening against multiple virus families using the same libraries by other members of this program will dramatically increase the likelihood of identifying antiviral compounds that are efficacious against a broad spectrum of agents. In order to develop drug candidates that exhibit antiviral activity against multiple members of the Alphavirus genus we propose the following specific aims:

Aim 1: Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block Alphavirus replication.

Rationale: Southern Research (SR) has developed and validated cell-based, high throughput assays for inhibitors of VEEV and CHIKV induced cytotoxicity. Initial use of this assay has already identified several compounds with antiviral activity against VEEV. Therefore, these assays will be employed to screen novel libraries of drugs that have not previously been screened against human pathogens including Alphaviruses.

Strategy: A CPE based assay will be used as a primary screen for antiviral compounds with activity against the VEEV and CHIKV. Following these initial screens, "hits" will be evaluated in dose response and cytotoxicity assays to determine compound-specific EC_{90}, CC_{90}, and selective indices.

Aim 2: Validate and characterize antiviral activity and off-target effects.

Rationale: Hit compounds identified in the primary screen could potentially affect any stage of virus replication; therefore, we will characterize the anti-Alphavirus compounds with regard to virus-specific efficacy and molecular mechanism of action.

Strategy: We will use a variety of secondary assays to identify: 1) breadth of anti-viral activity (test multiple Alphaviruses); 2) cell type-specificity (biologically relevant cells); 3) targets of antiviral compounds; and 4) ease of developing resistance phenotypes. Priority will be given to hits that are efficacious against many Alphaviruses and in multiple cell types, and do not affect virus entry or egress, nor activate IFN.

Aim 3:Chemical optimization and determination of *in vivo* efficacy of lead compounds.

Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit replication of diverse members of the Alphavirus genus. Chemical optimization of effective scaffolds should generate compounds with greater efficacy, selectivity and bioavailability.

Strategy: Hit compounds identified and characterized above will be triaged by the Medicinal Chemistry and Lead Development Core. Compounds with appropriate activity and pharmacokinetic properties will be evaluated using CHIKV and VEEV models of acute and persistent infection and disease.

Progress towards our goals is outlined for each Specific Aim:

SA1 HTS Screen of Novel Drug Libraries for Antiviral Compounds that Block Alphavirus Replication

1. 2015 Primary Screen: *VEEV HTS* identified 940 active samples and 8 out of 12 sent to OHSU had activity in NHDFs. *CHIKV HTS* identified 2,558 active compounds and 5 out of 11 were confirmed.
2. SR screened 347,000 compounds against $VEEV_{TC83}$ using Vero cells and 105 hits were identified. OHSU tested 35 and found 4 actives against CHIKV. SR derived analogs of two compounds (Tetralin-SR-33366 and Quinolone-SR-33394), which have been used for SAR and mode of action studies.
3. In order to both exclude compounds that block virus replication via activation of type I IFN responses and to enhance virus replication, Dr. DeFilippis constructed telomerized human foreskin fibroblast cells that lack IRF3 (THF-ΔIRF3). OHSU validated four anti-VEEV compounds as effective against CHIKV in these cells.
4. Construction and Sequencing of New CHIKV and VEEV Strains: The Alphavirus group has constructed new strains that will facilitate HTS and SAR including a new CHIKV strain expressing nano-Luciferase provided by UNC. Other recent isolates from Puerto Rico have been cloned and sequenced.
5. $VEEV_{TC83}$ has also been modified to encode nluc and is currently being validated at Colorado. $VEEV_{TC83}$-nLuc will be used by SR for SAR studies and the group for antiviral validation studies.

SA2. Validate and characterize antiviral activity and off-target effects

1. The group has developed multiple assays for secondary validation screens and to identify the mode of action for leads. To prevent duplication of effort and maximize experimental efficiency, each individual laboratory of the Alphavirus group has optimized specific assays.
2. **Quinolinones (SR-33394):** SR synthesized >90 analogs. OHSU tested the analogs in virus reduction assays and found the active compounds SR-33394 (EC_{90}=0.77μM), SR-34329 (EC_{90}=0.12μM), SRI-36506 (EC_{90}=4.9μM) and SR-36959 (EC_{90}=0.78μM). SR-34329 is active in VEE replicon assays indicating that the compound targets an early stage in virus replication. Colorado generated a $VEEV_{TC83}$ virus (NSP2 Y101C) that displays resistance to SR-34329. The mutation was reintroduced into the cDNA clone of VEEVTC83 to demonstrate that the single mutation confers resistance to SR-34329 and SR-33394. Antiviral mode of action (MOA) studies are underway for this chemical series.
3. **Tetralins-BenzoAnnulenes (SR-33366):** SR synthesized >125 analogs of SR-33366 for SAR. SR-34963 was found to have about a 10-fold increase in activity against CHIKV with an EC_{90}=0.45μM compared with SR-33366 (EC_{90}=3.2μM). Sequencing of UNC-derived resistance mutants identified changes in the NSP3 macrodomain, which is consistent with MOA studies showing that SR-34963 blocks viral RNA and protein synthesis. SR performed structural biology and modeling analysis and generated a 1.46Å resolution crystal structure of the nsp-3 macrodomain. Additional recent analogs show activity and are under SAR. SR-34963 is broadly active against alphaviruses (ONNV, MAYV, RRV, Una, and VEEV) as well as Flaviviruses (DENV and ZIKV). *In vivo* experiments with analog SR-36498 showed limited activity against CHIKV in mice, and further *in vivo* experiments are underway.
4. **VEEV 2015 HTS:** OHSU confirmed 8 of 12 active hits including: SR-36415 (IC_{90}=0.77μM), SR-36416 (IC_{90}=0.35μM), SR-36420 (IC_{90}=0.13μM), SR-36421 (IC_{90}=0.11μM), SR-36423 (IC_{90}=0.22μM), SR-36424 (IC_{90}=0.06μM), SR-36426 (IC_{90}=0.72μM), and SR-36427 (IC_{90}=0.25μM). SR-36426 and 27 were chosen for further SAR. Both work in $IRF3^{-/-}$ fibroblasts indicating that they do not function through IFN. SR-36426 is active against 5 different Alphaviruses and blocks infection prior to viral RNA synthesis. SR-36427 is active against VEEV and Mayaro virus and blocks infection after RNA synthesis. SR generated >50 SR-36427 analogs but none were shown to improve activity profile. Therefore, SR-36427 has been put on hold and a manuscript is in preparation describing antiviral activity for this chemical series. SAR for SR-36426 has shown promising results.
5. **CHIKV 2015 HTS:** OHSU confirmed 5 of 11 hits including: SR-33001 (IC_{90}=0.93μM), SR-35756 (IC_{90}=3.39μM), SR-35894 (IC_{90}=0.75μM), SR-36767 (IC_{90}=0.09μM), and SR-36768 (IC_{90}=0.23μM). Two compounds (SR-33001 and -36768) were active against 5 different Alphaviruses and SR-36767 was active against 4 Alphaviruses. SR-36767 blocks infection prior to RNA synthesis and is under assessment for MedChem. SR-33001 blocks viral replication at a step after viral RNA synthesis and >25 analogs have been synthesized with promising SAR results.
6. **Project 1, 2, 4 Hits:** DENV compound SR-37014 (IC_{90}=0.4μM) was active against CHIKV. SARS-CoV compounds SR-35742, -35894 and -36565 showed activity against VEEV but not CHIKV.

SA3. Chemical optimization and determination of *in vivo* efficacy of lead compounds

The group has developed a number of models to test *in vivo* efficacy of lead compounds. These include models of: 1) Acute CHIKV infection and joint disease and generation of a mouse-adapted CHIKV strain with enhanced replication and disease; 2) Intranasal inoculation of VEEV; 3) Chronic CHIKV infection and joint disease; 4) Lethal CHIKV and VEEV mouse models; and 5) CHIKV infection of NHP.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0410963140000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: UNIVERSITY OF COLORADO DENVER

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1. Dr	Thomas		Morrison		Co-Investigator	(b)(4) (b)(6)				28,375.00	7,945.00	36,320.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **36,320.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
1	Post Doctoral Associates	(b)(4)			11,875.00	2,256.00	14,131.00
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
1	PRA				21,054 00	5,895 00	26,949 00
2	**Total Number Other Personnel**					**Total Other Personnel**	**41,080.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**77,400.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0410963140000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF COLORADO DENVER

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	0.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**0.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0410963140000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: UNIVERSITY OF COLORADO DENVER

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1. Materials and Supplies	30,550 00
2. Publication Costs	0 00
3. Consultant Services	0.00
4. ADP/Computer Services	0.00
5. Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. Animal Costs	40,000.00
Total Other Direct Costs	**70,550.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**147,950.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. MTDC	55.5	147,950.00	82,112.00
		Total Indirect Costs	**82,112.00**

Cognizant Federal Agency DHHS, Arif Karim, 415-437-7820
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**230,062.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: Morrison_BudgJusty.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION

Personnel ($77,400)

Thomas E. Morrison, Ph.D., Co-Investigator (b)(4) **months).** Dr. Morrison has extensive experience and expertise in alphavirus pathogenesis, host immune responses to virus infection, and mouse models. Dr. Morrison will be responsible for the overall administration and direction of the project. He will be responsible for the overseeing studied designed to determine the mechanism of action of select compounds and for the testing of candidate therapeutics for their ability to protect mice from chronic CHIKV infection and disease. He will work in close collaboration with Drs. Heise, DeFilippis and Streblow, as well as the other research project leaders to set priorities for which drugs will be evaluated for mechanism of action and which will be tested within the CHIKV chronic disease model.

Katie Carpentier, Post-Doctoral (b)(4) **months).** Ms. Carpentier will perform mechanism of action studies in cells for select compounds. Ms. Carpentier also will perform CHIKV inoculations of mice, administer therapeutics, perform animal necropsies, and process tissues for quantification of CHIKV RNA and for the assessment of tissue pathology.

Nick May, Professional Research Assistant (b)(4) **months).** Mr. May will assist Mr. Hawman in testing therapeutics for their ability to protect mice from chronic CHIKV infection and disease. He will perform real time PCR analysis to evaluate viral RNA loads in virally infected tissues in the presence or absence of candidate therapeutics. In addition, Mr. May will perform mechanism of action studies in cells for select compounds.

Other significant contributors

Stephanie Montgomery, Ph.D., D.V.M., North Carolina State University, Raleigh, NC (0 person months). Dr. Montgomery and Dr. Morrison have an active collaboration related to CHIKV-induced tissue pathology that was an important component of two previous publications. Dr. Montgomery, a veterinary pathologist with a doctorate in alphavirus biology, will provide expert analysis of histopathological changes in murine tissues.

Supplies ($30,550)

The evaluation of candidate therapies for the treatment of chronic CHIKV infection and disease in the mouse model requires stocks of infectious CHIKV. Therefore, we request funds for tissue culture consumables and transcription kits. To evaluate the effects of candidate therapies requires the isolation of RNA from specific tissues and the quantification of CHIKV RNA via qRT-PCR and preparation of tissues for evaluation of histopathologic changes. Therefore funds are requested to cover the costs of consumables such as Trizol, RNA isolation kits, reverse-transcriptase enzyme, and PCR reagents. We will also need to generate H & E stained tissue section for evaluation of histopathologic changes. In addition, we request funds for reagents and fees associated with high-throughput sequencing of any identified resistance mutant that emerge out of the animal experiments. Lastly, since some assays will need to be performed under BSL-3 conditions, funds are requested to cover the cost of personal protective equipment, such as gloves and tyvek suits.

Animals ($40,000)

The CHIKV chronic infection model utilizes three-to-four week old C57BL/6 mice. Therefore, we request funds to cover costs associated with the purchase of breeding pairs of mice, costs associated with maintaining an active breeding colony, and costs associated with housing experimental mice for up to 4-6 months under ABSL3 conditions.

A. COMPONENT COVER PAGE

Project Title: Project 4.2 Identification and characterization of novel drugs that target the influenza virus polymerase functions

Component Project Lead Information:

(b)(6), (b)(3) 7 U S C § 8401

B. COMPONENT ACCOMPLISHMENTS

B.1 WHAT ARE THE MAJOR GOALS OF THE PROJECT?

The overall goal of this project is to identify new therapies that target influenza virus replication The global health burden of annual influenza epidemics coupled with the emergence of highly pathogenic strains of influenza virus has highlighted the urgent need for new effective treatments A primary concern with the current drugs (amantadines and neuraminidase inhibitors) used to treat influenza is the development of resistance mutations that negate therapeutic benefit. Published evidence suggests that targeting the influenza virus RNA dependent RNA polymerase (RdRp) is a rational approach for antiviral therapy The RdRp is responsible for a number of functions including 5'cap recognition, endonuclease activity, replication, transcription, and polyadenylation Recently, cryo-EM reconstitution studies identified branched-ribonucleoproteins (RNPs) structures as putative replication intermediates and suggested a mechanism for viral replication by a second polymerase activity on the RNP template [1]. The second polymerase activity is believed to be a function of the polymerase complex Clearly, the RdRp provides multiple functional domains that could be targets for antiviral drug therapy Previous studies showed that mutations in the conserved regions of PB1 subunit of the polymerase complex produce inactive RNA polymerase [2]. We hypothesize that compounds that specifically target the polymerase complex might reduce the frequency of escape mutations, or promote escape mutants that are unfit for replication We have recently identified potential hit compounds from previous HTS screens that significantly inhibit the influenza virus polymerase activity in an RdRp transient assay. These hit compounds were effective against three different strains of influenza viruses in CPE assays. Between Southern Research (SR) and the University of Alabama at Birmingham (UAB), all the necessary primary and secondary assays to perform HTS screening and identify compounds that specifically target the influenza virus polymerase activity have been developed We propose the following specific aims:

Aim#1. Employ a validated HTS primary assay to screen novel drug libraries for antiviral compounds that specifically block influenza virus replication.
Hypothesis and rationale We hypothesize that by targeting the polymerase complex, we might reduce the frequency of mutational evasion because the mutants will be unfit for replication Recent studies demonstrated that the nucleoside inhibitor T-705 induces lethal mutagenesis in H1N1 viruses in vitro resulting in a nonviable phenotype [3]. Targeting the influenza polymerase activity might prove more effective than targeting the viral glycoproteins because there are multiple proteins, as well as protein. protein and protein. RNA interactions, which could be targeted. Our goal is to identify compounds against the conserved regions of influenza virus polymerase subunits that might be effective against multiple viral strains.
Experimental strategy The proposed transient influenza polymerase assay in aim#2 to identify anti-polymerase hits is not adaptable for HTS, and therefore a CPE-based assay will be used as a primary assay to screen novel libraries against influenza viruses. We will screen libraries that have not been previously screened for activity against the viruses covered in this proposal. These libraries are composed of highly diversified small molecules that contain novel and original drug-like features with distinct topologies and diverse functionalities.

Aim#2: Characterize the antiviral activity of hit compounds and identify anti-polymerase inhibitors
Hypothesis and rationale: The existing hit compounds with polymerase inhibitory activity might target one or more subunits of the influenza virus polymerase. The CPE-based HTS screening will identify additional hit compounds that target all stages of the virus life cycle, including multiple functional domains of the influenza RNA polymerase. We have designed an experimental strategy that will focus our analysis on the hit compounds that block post-entry steps of viral infection.
Experimental strategy We will use a variety of secondary assays to identify compounds that specifically inhibit the functions of the viral polymerase complex. Our proposed secondary assays will identify and exclude hit compounds that target viral entry and release, as well as interferon inducers. Following this exclusion process we will examine the remaining positive hit compounds in the transient polymerase assay. Once compound specificity for the viral polymerase is demonstrated, tertiary assays will be performed to determine the target within the polymerase complex

Aim#3: Chemical optimization and determination of the in vivo efficacy of lead compounds.
Hypothesis and Rationale: Our secondary assay characterization is expected to identify multiple compounds that specifically inhibit the influenza replication complex. Chemical optimization of the effective scaffolds should generate compounds with greater efficacy, selectivity, and bioavailability.
Experimental strategy The hit compounds from the HTS will be triaged and progressed as outlined in the Chemistry core Compounds with the appropriate activity and pharmacokinetic properties will be evaluated using in-house mouse infection models

B.1.a Have the major goals changed since the initial competing award or previous report?

No

B.2 WHAT WAS ACCOMPLISHED UNDER THESE GOALS?

File uploaded. B2 Project 4.1 SD 12.12.pdf

B.3 COMPETITIVE REVISIONS/ADMINISTRATIVE SUPPLEMENTS

Not Applicable

B.4 WHAT OPPORTUNITIES FOR TRAINING AND PROFESSIONAL DEVELOPMENT HAS THE PROJECT PROVIDED?

File uploaded B4 for Project 4.2 pdf

B.5 HOW HAVE THE RESULTS BEEN DISSEMINATED TO COMMUNITIES OF INTEREST?

NOTHING TO REPORT

B.6 WHAT DO YOU PLAN TO DO DURING THE NEXT REPORTING PERIOD TO ACCOMPLISH THE GOALS?

The initial 892 hits (≥ 50% inhibition in the antiviral assay with cell viability ≥ 80%) identified from the ELVIRA HTS campaign were counter screened in the Mirrorball M2 Immunofluorescence assay (IFA) against H3N2 virus This resulted in 158 compounds that had ≥ 50% IAV inhibition with cell viability ≥ 80%. These compounds were triaged by structural analysis to eliminate those containing undesirable chemical properties which provided 17 compounds In addition, as recommended by the advisory committee, an additional 21,000 SR proprietary compounds were evaluated in H3N2 CPE assay using MDCK cells which resulted in an additional 3 confirmed hits As in other programs, fresh samples, will be acquired, analyzed (purity) and retested in the IFA to confirm activity as well as counter-screened for cytotoxicity effect in the A549 host cells and a CC50 value will be determined The compounds which reconfirm will subsequently be evaluated in the RdRp assay for polymerase inhibition as well as for neuraminidase, hemagglutinin and viral entry inhibition. From these compounds, it is expected that some compounds will be identified which are polymerase inhibitors and potentially compounds that have antiviral activity, but not through any of the known mechanisms. If compounds with this profile are identified, they will be moved into studies to generate resistant mutants, whose genomes will be sequenced to identify the other potential viral targets. From these studies, we will obtain compounds that will move to the Chemistry Core. New compounds that are synthesized will be evaluated in the IFA assay (SAR driving assay) and compounds with EC50 ≤ 20 μM will be tested in the RdRp assay Active compounds will be selected for preliminary structure-activity relationship studies by the Chemistry Core Compounds meeting the established criteria in the IFA and VTR assays will then be tested in primary human small airway epithelial cells using the NanoLuc influenza PATSN (H1N1).

With respect to future in vivo studies, the in vivo team purchased and documented accuracy of a microrectal thermometer for measuring body temperatures of mice as required by IACUC for influenza studies. Lethality studies will be performed to determine the optimal dose for viral intranasal infections in BALB/c mice. Using the NanoLuc reporter IAV, we will perform imaging of infected mice through the UAB Imaging Core to track viral distribution in vivo in the presence or absence of the selected inhibitors. Subsequently, the efficacy and toxicity of lead compounds will be evaluated.

B.2 *What was accomplished under these goals?*

The HTS screening campaign carried out in the third quarter of Year 3, using the ELVIRA cell reporter assay, identified 892 positive hit compounds with confirmed antiviral activity in a dose dependent manner. During the course of this year these compounds were further evaluated for reconfirmation using several independent assays described below.

Virus yield reduction assays

The 892 compounds were evaluated further in a 384-well CPE assay in MDCK cells at concentrations of 10 and 2 μM against A/CA/10/2009 and A/Panama/2001/99 including a cytotoxicity assay with equivalent compound exposure. All data were transferred to the HTS group to upload into the SRI database to identify the compounds that were active against both strains of the virus. Further studies characterized the confirmed hits with a virus yield reduction assay. A total of 180 tests were performed against A/CA/10/2009 (H1N1), A/Panama/2001/99 (H3N2), and/or B/Florida/4/2006. These studies identified 7 confirmed hits with EC_{90} values <20 μM for H1N1 and H3N2 strains.

Immunofluorescence assay using mirrorball technology

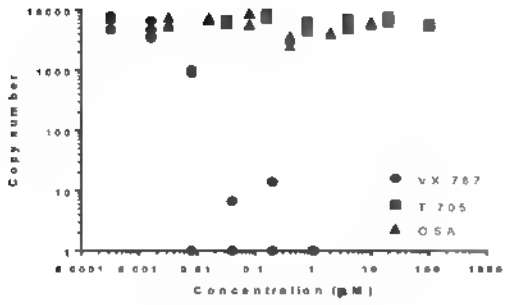
Historically, the CPE assay has resulted in low numbers of confirmed hits and inconsistent results. Therefore, we developed and implemented a new assay for reconfirmation. Thus, an immunofluorescence assay (IFA) using the mirrorball technology and human A549 lung epithelial cells and an antibody directed against the M2 protein of IAV was used to rescreen the 892 compounds found in the primary HTS. From these 892 compounds, 158 compounds showed antiviral activity using IFA with IC_{50} < 50 μM and no cytotoxicity (CC_{50} > 40 μM) on A549 cells. The structures of these compounds are currently being evaluated for further investigation.

Screening of a subset of SR compounds

To identify additional hits, 20,530 compounds from the SR proprietary collection were screened in the MDCK CPE assay. From this set of compounds, 2,155 compounds were tested in a concentration response assay from which six compounds previously known to have anti-viral effects for influenza were identified. An additional 223 active compounds were identified from the remaining 18,275 compounds which were tested at a single concentration. These compounds were retested for a concentration dependent response to confirm activity in the anti-viral CPE assay and were also counter-screened for cytotoxicity against MDCK cells. Thirty-three (33) were confirmed with IC_{50} < 50 μM and a corresponding CC_{50} > 40 μM. These compounds are currently being counter-screened in the ELVIRA reporter assay and the IFA assay to further validate the anti-viral effect.

Development of an RdRp endonuclease assay.

Previous studies with a 384-well assay using NanoLuc influenza strain A/California/04/2009 pdm (H1N1) PATSN in primary human small airway epithelial cells identified a number of compounds with antiviral activity. To further characterize these compounds, a 96-well qPCR assay was developed to assess the inhibition of endonuclease activity of the RdRP complex. The assay specifically detects the formation of chimeric mRNAs that result from the endonucleolytic cleavage of the cellular capped RNAs that are subsequently used to initiate RNA transcription of influenza genes. Specifically, the qPCR assay detects chimeric RNAs that contain the 5' cap and the first 11 base pairs from the U2 snRNA and the influenza PB2 RNA. This assay is unique because it can specifically measure chimeric RNAs formed in the context of antiviral with the native RdRP and native substrates. This assay correctly identified the EC_{50} of the cap binding inhibitor VX-787 and did not detect activity from either T-705 or ribavirin (see graph below). This assay will be further refined and used to identify novel inhibitors of the endonuclease function of the RdRP.


Copy number
10000
1000
100
10
1
VX 787
T 705
OSA
Concentration (µM)

B.4 *What opportunities for training and professional development has the project provided?*

Postdoctoral Fellows are active in the project. Southern Research has developed a comprehensive and strategic career Individual Development Plan (IDP) for Post-Doctoral Fellows within the Drug Discovery Division. Our training program is aimed at developing knowledge, skills, and reputation and promoting the advancement of an independent career in drug discovery against infectious diseases. To ensure that trainees receive the knowledge and skills that are the necessary foundation of a scientific career, we offer training that gives them exposure to multiple key areas with which our faculty have established expertise that includes but is not limited to: (a) biology and pathogenesis of infectious agents, (b) identification of novel drug targets, (c) development of pertinent assays for drug discovery, identification and development of molecular-targeted therapeutics, and (d) mechanism of action of drugs. Seminar series, journal clubs, manuscript and grant writing guidance ensure that each trainee will receive exposure to each of these areas (all of which are mainstays in the Department of Infectious Diseases). Likewise, the qualifications of our staff ensure that expertise will be adequate to offer guidance that is both current and accessible. Additionally, each trainee is strongly encouraged to submit at least one abstract annually for presentation at a national meeting which relates to his/her research areas. Progress of each trainee is evaluated on an annual basis. These evaluation meetings are used for defining key objectives and goals for progress for the upcoming year.

C. COMPONENT PRODUCTS

C.1 PUBLICATIONS

Not Applicable

C.2 WEBSITE(S) OR OTHER INTERNET SITE(S)

Not Applicable

C.3 TECHNOLOGIES OR TECHNIQUES

NOTHING TO REPORT

C.4 INVENTIONS, PATENT APPLICATIONS, AND/OR LICENSES

Not Applicable

C.5 OTHER PRODUCTS AND RESOURCE SHARING

Nothing to report

D. COMPONENT PARTICIPANTS

Not Applicable

E. COMPONENT IMPACT

E.1 WHAT IS THE IMPACT ON THE DEVELOPMENT OF HUMAN RESOURCES?

Not Applicable

E.2 WHAT IS THE IMPACT ON PHYSICAL, INSTITUTIONAL, OR INFORMATION RESOURCES THAT FORM INFRASTRUCTURE?

Not Applicable

E.3 WHAT IS THE IMPACT ON TECHNOLOGY TRANSFER?

NOTHING TO REPORT

E.4 WHAT DOLLAR AMOUNT OF THE AWARD'S BUDGET IS BEING SPENT IN FOREIGN COUNTRY(IES)?
Not Applicable

F. COMPONENT CHANGES

F.1 CHANGES IN APPROACH AND REASONS FOR CHANGE

Not Applicable

F.2 ACTUAL OR ANTICIPATED CHALLENGES OR DELAYS AND ACTIONS OR PLANS TO RESOLVE THEM

NOTHING TO REPORT

F.3 SIGNIFICANT CHANGES TO HUMAN SUBJECTS, VERTEBRATE ANIMALS, BIOHAZARDS, AND/OR SELECT AGENTS

F.3.a Human Subjects

No Change

F.3.b Vertebrate Animals

No Change

F.3.c Biohazards

No Change

F.3.d Select Agents

No Change

G. COMPONENT SPECIAL REPORTING REQUIREMENTS

G.1 SPECIAL NOTICE OF AWARD TERMS AND FUNDING OPPORTUNITIES ANNOUNCEMENT REPORTING REQUIREMENTS

Not Applicable

G.2 RESPONSIBLE CONDUCT OF RESEARCH

Not Applicable

G.3 MENTOR'S REPORT OR SPONSOR COMMENTS

Not Applicable

G.4 HUMAN SUBJECTS

G.4.a Does the project involve human subjects?

No

G.4.b Inclusion Enrollment Data

Not Applicable

G.4.c ClinicalTrials.gov

Not Applicable

G.5 HUMAN SUBJECTS EDUCATION REQUIREMENT

Not Applicable

G.6 HUMAN EMBRYONIC STEM CELLS (HESCS)

Does this project involve human embryonic stem cells (only hESC lines listed as approved in the NIH Registry may be used in NIH funded research)?

No

G.7 VERTEBRATE ANIMALS

Not Applicable

G.8 PROJECT/PERFORMANCE SITES

Not Applicable

G.9 FOREIGN COMPONENT

Not Applicable

G.10 ESTIMATED UNOBLIGATED BALANCE

Not Applicable

G.11 PROGRAM INCOME

Not Applicable

G.12 F&A COSTS

Not Applicable

RESEARCH & RELATED BUDGET - SECTION A & B FINAL

ORGANIZATIONAL DUNS*: 0069005260000

Budget Type*: ● Project ○ Subaward/Consortium

Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

A. Senior/Key Person

	Prefix	First Name*	Middle Name	Last Name*	Suffix	Project Role*	Base Salary ($)	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits ($)*	Funds Requested ($)*
1.		(b)(6) (b)(3) 7 U S C § 8401				Project Leader	0.00	(b)(4), (b)(6)			14,650.00	6,256.00	20,906.00

Total Funds Requested for all Senior Key Persons in the attached file

Additional Senior Key Persons: File Name:

Total Senior/Key Person **20,906.00**

B. Other Personnel

Number of Personnel*	Project Role*	Calendar Months	Academic Months	Summer Months	Requested Salary ($)*	Fringe Benefits*	Funds Requested ($)*
	Post Doctoral Associates						
	Graduate Students						
	Undergraduate Students						
	Secretarial/Clerical						
5	2 Biologist, 1 Life Sci Res., 1 Compd Mgr, 1 Scientist	(b)(4)			144,156 00	61,501.00	205,657.00
5	**Total Number Other Personnel**					**Total Other Personnel**	**205,657.00**
						Total Salary, Wages and Fringe Benefits (A+B)	**226,563.00**

RESEARCH & RELATED Budget {A-B} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTION C, D, & E

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

C. Equipment Description	
List items and dollar amount for each item exceeding $5,000	
Equipment Item	**Funds Requested ($)***
Total funds requested for all equipment listed in the attached file	**0.00**
Total Equipment	**0.00**

Additional Equipment: File Name:

D. Travel	Funds Requested ($)*
1. Domestic Travel Costs (Incl. Canada, Mexico, and U.S. Possessions)	10,250.00
2. Foreign Travel Costs	0.00
Total Travel Cost	**10,250.00**

E. Participant/Trainee Support Costs		Funds Requested ($)*
1. Tuition/Fees/Health Insurance		0 00
2. Stipends		0.00
3. Travel		0 00
4. Subsistence		0.00
5. Other:		
0 Number of Participants/Trainees	**Total Participant Trainee Support Costs**	**0.00**

RESEARCH & RELATED Budget {C-E} (Funds Requested)

RESEARCH & RELATED BUDGET - SECTIONS F-K

ORGANIZATIONAL DUNS*: 0069005260000
Budget Type*: ● Project ○ Subaward/Consortium
Enter name of Organization: SOUTHERN RESEARCH INSTITUTE

Start Date*: 03-01-2018 **End Date*:** 02-28-2019

F. Other Direct Costs	Funds Requested ($)*
1 Materials and Supplies	116,348 00
2 Publication Costs	0 00
3 Consultant Services	0.00
4 ADP/Computer Services	0.00
5 Subawards/Consortium/Contractual Costs	0.00
6. Equipment or Facility Rental/User Fees	0.00
7. Alterations and Renovations	0.00
8. BSL Fees/Differential	8,200.00
Total Other Direct Costs	**124,548.00**

G. Direct Costs	Funds Requested ($)*
Total Direct Costs (A thru F)	**361,361.00**

H. Indirect Costs

Indirect Cost Type	Indirect Cost Rate (%)	Indirect Cost Base ($)	Funds Requested ($)*
1. OH - Salaries and Benefits	120.0	226,563.00	271,877.00
2. G&A - Total Direct Cost + OH	20.0	633,237.00	126,647.00
3. CFC - Salaries and Benefits	7.3	226,563.00	16,539.00
4. CFC - Total Direct Cost + OH	1.0	633,237.00	633.00
		Total Indirect Costs	**415,696.00**

Cognizant Federal Agency DHHS, Steven Zuraf, 301-492-4855
(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs	Funds Requested ($)*
Total Direct and Indirect Institutional Costs (G + H)	**777,057.00**

J. Fee	Funds Requested ($)*
	0.00

K. Budget Justification* File Name: BUDGET JUSTIFICATION-Project 4 Year 5.pdf
(Only attach one file.)

RESEARCH & RELATED Budget {F-K} (Funds Requested)

BUDGET JUSTIFICATION – Southern Research Institute

Section A – Personnel - Biochemistry and Molecular Biology

(b)(6), (b)(3) 7 U S C § 8401 Ph.D. (b)(4) months for Year 5), is a (b)(6), (b)(3) 7 U S C § 8401 (b)(6), (b)(3) 7 U S C § 8401 working in the fields of virology and bacteriology (b)(6), (b)(3) 7 was one of the developers of the live attenuated *Vibrio cholerae* vaccine 638, which is currently in clinical trials and has been shown to be well tolerated and immunogenic in humans. (b)(6), (b)(3) 7 also discovered a new mechanism of horizontal transmission of the cholera toxin genes mediated by the filamentous bacteriophage VGJφ in V. cholerae and has made other outstanding contributions to the field of filamentous phage biology. After (b)(6), joined Southern Research he has been involved in several projects not only in the field of bacteriology, but also in virology, specifically in the study of the mechanism of entry of Ebola virus and the designing and development of a new assay for identifying inhibitors of the VEEV protease. In this last field (b)(6), (b)(3) 7 is currently focused in the discovery of antiviral compounds against influenza less prone to develop viral resistance by targeting the RNA polymerase (b)(6), (b)(3) 7 U S C § 8401 will supervise and perform secondary confirmatory assays on hit compounds found in the primary HT screening against IAV, specifically by implementing a qRT-PCR that has been optimized in his lab (b)(6), (b)(3) 7 will also supervise secondary assays for mechanistic characterization. In particular, cell entry assays to exclude hit compounds that act on viral entry (b)(6), (b)(3) 7 will assist in the designing of experiments to these aims and presentation of data and manuscripts, as well as participate in Project meetings.

(b)(6), (b)(3) 7 U S C § 8401 a Biologist in (b)(6), (b)(3) 7 U S C § 8401 laboratory (b)(4) months for Year 5) will be responsible for growing and determining the titer of influenza virus stocks to make them available in downstream confirmatory assays and mode of action studies (Aim#1 & 2). She will also conduct secondary screening of hit compounds against different influenza virus subtypes (b)(6), (b)(3) 7 U S C § 8401 will also perform antiviral biochemical assays related to Aim#1 & 2, and will assist with BSL-3 evaluations of antiviral compounds for broad efficacy (Aim#2). She will also perform other biochemical assays including hemaglutination, and neuramindase assays.

(b)(6), (b)(3) 7 U S C § 8401 Ph.D., a (b)(6), (b)(3) 7 U S C § 8401 researcher in (b)(6), (b)(3) 7 U S C § 8401 laboratory (b)(4) (b)(4) months for Year 5) will conduct secondary screening of hit compounds against an influenza virus *in vitro* polymerase assay (Aim#2d) (b)(6), (b)(3) 7 will also perform the plaque reduction assays (Aim#2c) and the interferon assays (Aim#2b). Additionally, (b)(6), (b)(3) 7 U S C § 8401 will perform experiments to determine the effect of hit compound on influenza virus protein expression (Aim#2f) and RNA expression (Aim#2e).

(b)(6), (b)(3) 7 U S C § 8401 (b)(6), (b)(3) 7 U S C § 8401 in (b)(6), (b)(3) 7 U S C § 8401 laboratory (b)(4) months for Year 5) will continue developing and optimizing cell entry assays for influenza, west Nile, dengue, and SARS viruses (Aim#2a) and the interferon inhibition assay (Aim 2b) (b) 6), (b) 3) 7 will also assist in screening the hit compounds in the entry assays, and in implementation of the qRT-PCR confirmatory assay for Influenza viruses.

(b)(6), (b)(3) 7 U S C § 8401 (b)(6), (b)(3) 7 U S C § 8401 M.S., (b)(4) months for year 5) will be responsible for compound management and drugging for the biological assays.

(b)(6), (b)(3) 7 U S C § 8401, oversees the HTS informatics group (b)(4) months for year 5) and will be responsible for writing the data templates for the screening effort and data import and analysis and depositing the data with Enterprise Content Management Documentum CenterStage database (b)(6) (b)(3) 7 U S C § 8401 manages our ActivityBase software, the Oracle database, and will facilitate transfer of data between the groups including the cheminformatics staff.

Year 5 costs:

These include labor and materials for cell culture, biochemical assay performance, and general supply costs. BSL-3 laboratory charges are included for 18 days of operations and incubations. We request travel for 2 Project Leaders and 2 staff members to one scientific meeting per year to present our findings.

Supplies	$113,870
BSL-3 charges	$8,000
Travel	$10,000

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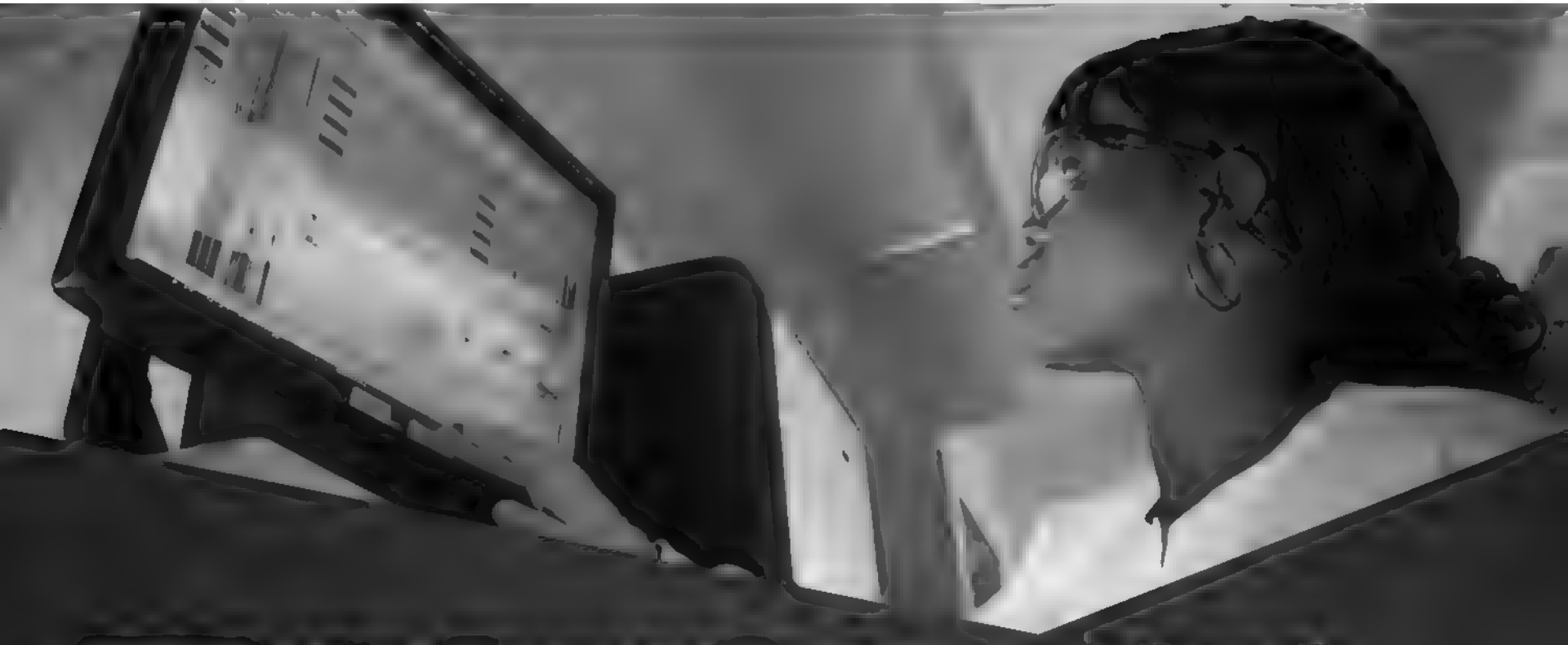

DCR/Leidos Financial Review of Ebola and HIV Projects in West Africa

June 17, 2019

Agenda: DCR/Leidos Financial Review of Ebola and HIV in West Africa
Monday, June 17, 2019 2:00pm - 3:30pm

Non-responsive

Non-responsive **Purpose: To provide DCR senior leadership an overview of funding versus actuals for the clinical research program in West Africa and to discuss/strategize on the following:Projected expenditures based on current funding, studies and program initiatives** Non-responsive

Non-responsive

Non-responsive

Agenda:Introduction & Agenda
Current Study StatusStudy Timelines Non-responsive

Non-responsive

June 2019 Studies Current Status

Study	Date/version	Status of recruitment	Study POP	Enrollment status (as of 6/13/2019)	Study Site(s)	Items of note
Non-responsive						
PREVAIL IV (Gilead)	May 13, 2019/v10.0	Open enrollment	Jul 2016- Jan 2021	38/60	JFK, C.H. Rennie, Duport- Liberia, Maf - Guinea	Open in the event a positive semen is idnetified from P3; but currently no participants actively on study. Open in the event the study is initiated in the DRC following the current outbreak
Non-responsive						

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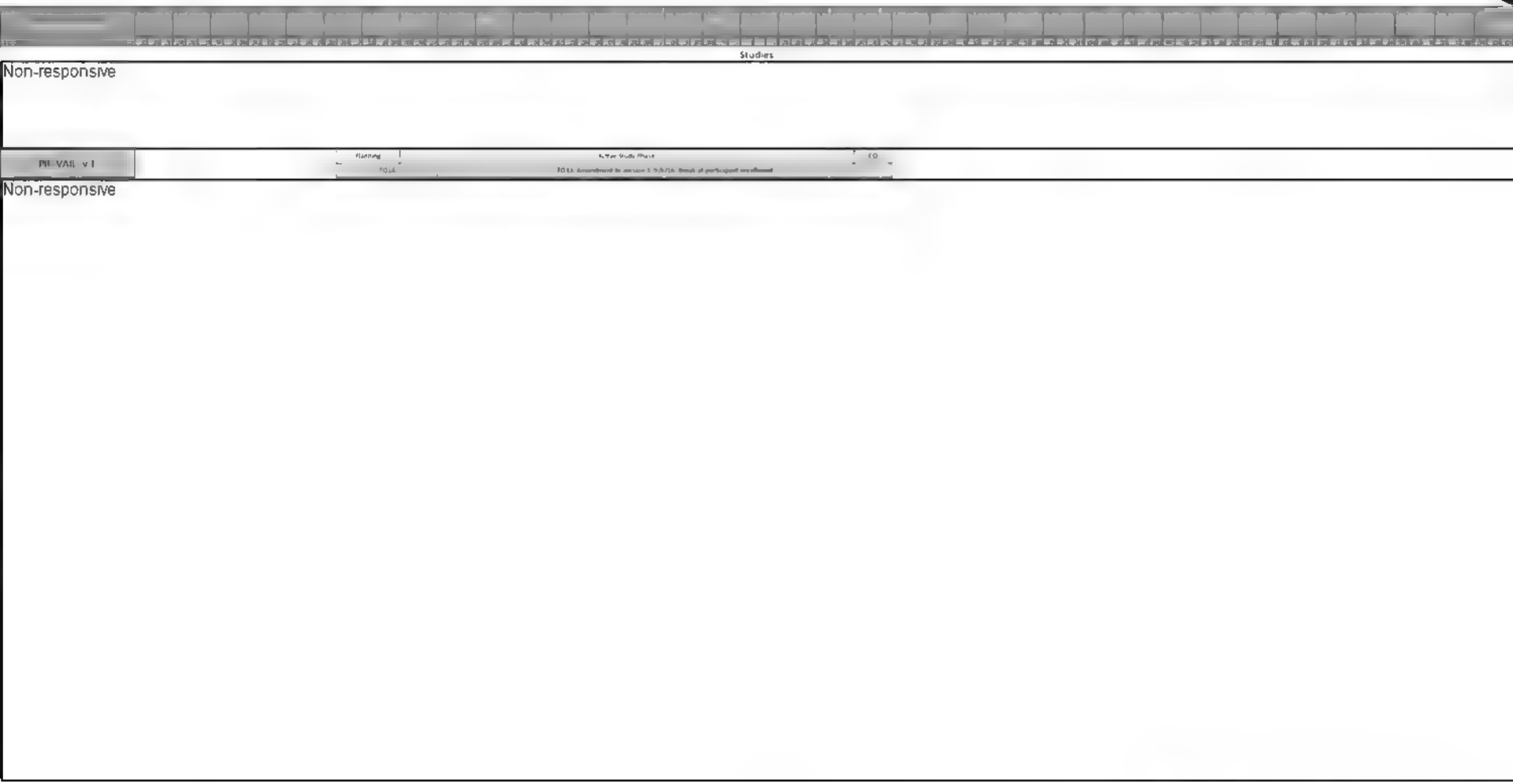
Non-responsive

Strategy Map



Strategy Map Continued

Summary of Leidos Ebola Budget: FY15-FY23

Leidos Non-Severable Funding

Ebola Funding for West Africa	Actual FY14	Actual FY15	Actual FY16	Actual FY17	Actual FY18	Projection FY19	Projection FY20	Projection FY21	Projection FY22	Projection FY23	Projection FY24	Projection FY25	Total
Non-responsive													
ncremental Cost Per Study													
Non-responsive													
PREVAIL IV - Liberia	-	-	571,442	659,020	388,268	187,070	-	-	-	-	-	-	1,805,800
PREVAIL IV - Guinea	-	-	-	99,078	1,288,933	100,054	-	-	-	-	-	-	1,488,064
Non-responsive													
MCM RCT in DRC					8,170	15,567,827	4,551,113						20,127,110
Non-responsive													

*No Fund ng availble past FY23

Summary of Leidos Subcontractor Estimates

PO#	Subcontractor	Study	FY16	FY17	FY18	FY19 EAC	FY20	FY21	FY22	FY23	Total	Funding Source
Non-responsive												
16X054Q5	The University of Minnesota	PREVAIL IV Liberia	71,221	426,964	289,608	145,492	-	-			933,285	TO 14/33
16X054Q5	The University of Minnesota	PREVAIL IV Guinea			205,925	71,075					277,000	TO 43
Non-responsive												
16X055Q	The Mitchell Group	PREVAIL IV Guinea			174,434	13,872					188,306	TO 43
Non-responsive												
DRC Subcontracts												
18X107CQ1	The University of Minnesota	MCM-RCT				341,998	97,714				439,712	TO 43, 59
16X055Q	The Mitchell Group	MCM-RCT				354,839	-	-			354,839	TO 43
18X107EQ1	The Mitchell Group	MCM-RCT				9,915,214	4,283,478	-			14,198,692	TO 43, 59
Non-responsive												
18X107HT1	FHI 360	MCM-RCT				121,642	-	-			121,642	TO 43
18X107AT1	ALMA	MCM-RCT				122,726					122,726	TO 43
16X078Q	Prudence Social Mobilometrics	MCM-RCT				120,263	40,088				160,351	TO 43, 59
		Subtotal-DRC	$	$	$	$ 11,027,089	$ 4,431,015	$ 9,735	$ 9,735	$ 9,735	$ 15,487,309	
Non-responsive												

Ebola Funding Highlights

Non-responsive

Ebola Funding Highlights

Funding Mechanism	Status/Updates
TO 43TO 59	Task Order 43 Non-responsive ends in FY22 and will be fully spentPoP: Non-responsive Non-responsive Funding MCM-RCT in the Non-responsive Non-responsive Task Order 59 Non-responsive ends in FY23 and will be fully spent Non-responsive Non-responsive Will fund MCM-RCT in DRC in FY20 Non-responsive Non-responsive Non-responsive

In Summary:

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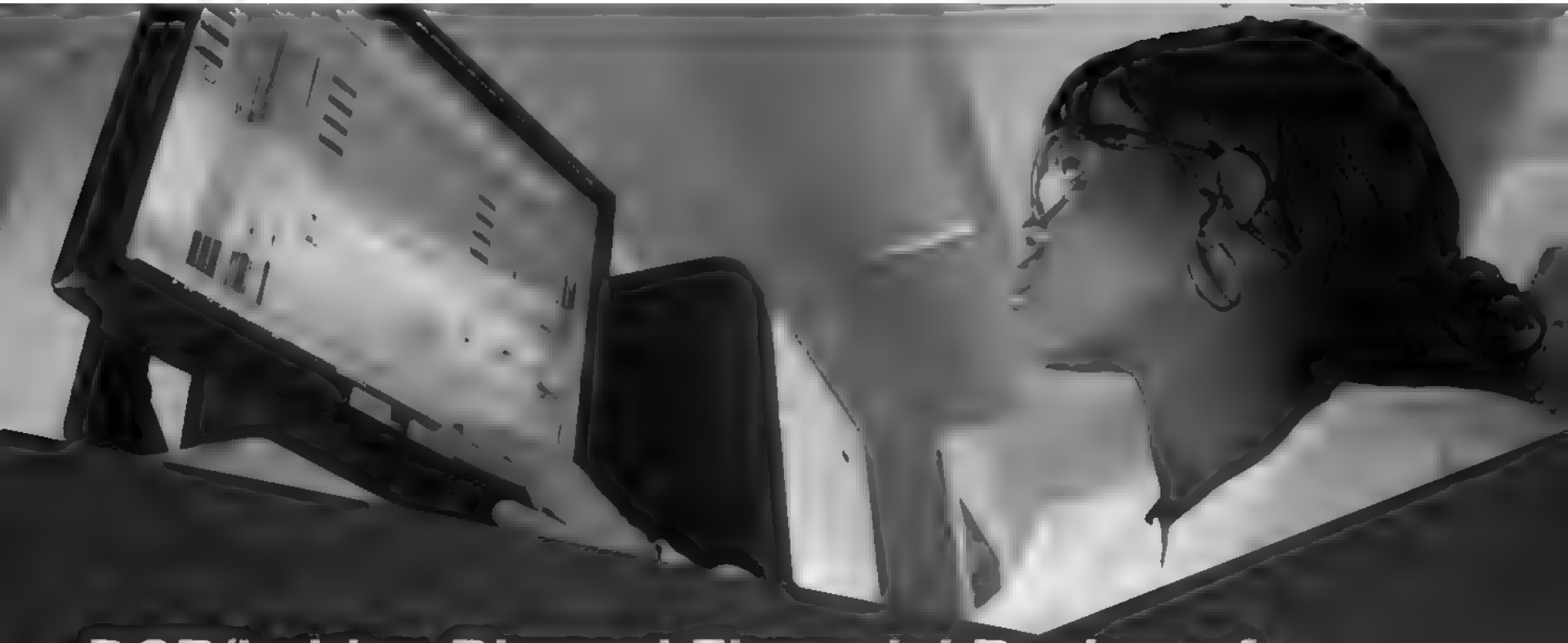


DCR/Leidos Biomed Financial Review of Ebola and HIV Projects in West Africa & the DRC

October 8, 2019

DEPARTMENT OF HEALTH AND HUMAN SERVICES | National Institutes of Health | National Cancer Institute

Frederick National Laboratory for Cancer Research sponsored by Leidos Biomedical Research, Inc. for the National Cancer Institute

Agenda: DCR/Leidos Biomed Financial Review of Ebola and HIV in West Africa & the DRC Tuesday, October 8, 2019 2:00pm - 3:00pm

Attendees: Cliff Lane, Laura McNay, Beth Grace, Beth Baseler, Laurie Lambert, Calvin Proffitt, Matt Hohn, Sara Albert, Ben MartinezOn the line: Lou Hepp, Rodney Ritenour, John Thomas, Denise Motok, Theresa EngelPurpose: To provide DCR senior leadership an overview of funding versus actuals for the clinical research program in West Africa and the DRC and to discuss/strategize on the following:Projected expenditures based on current funding, studies and program initiatives Non-responsive

Non-responsive

Agenda:Introduction & Agenda Current Study StatusStudy TimelinesSummary of FundingFinancial Executive Summary - Ebola Non-responsive

Non-responsive

Non-responsive

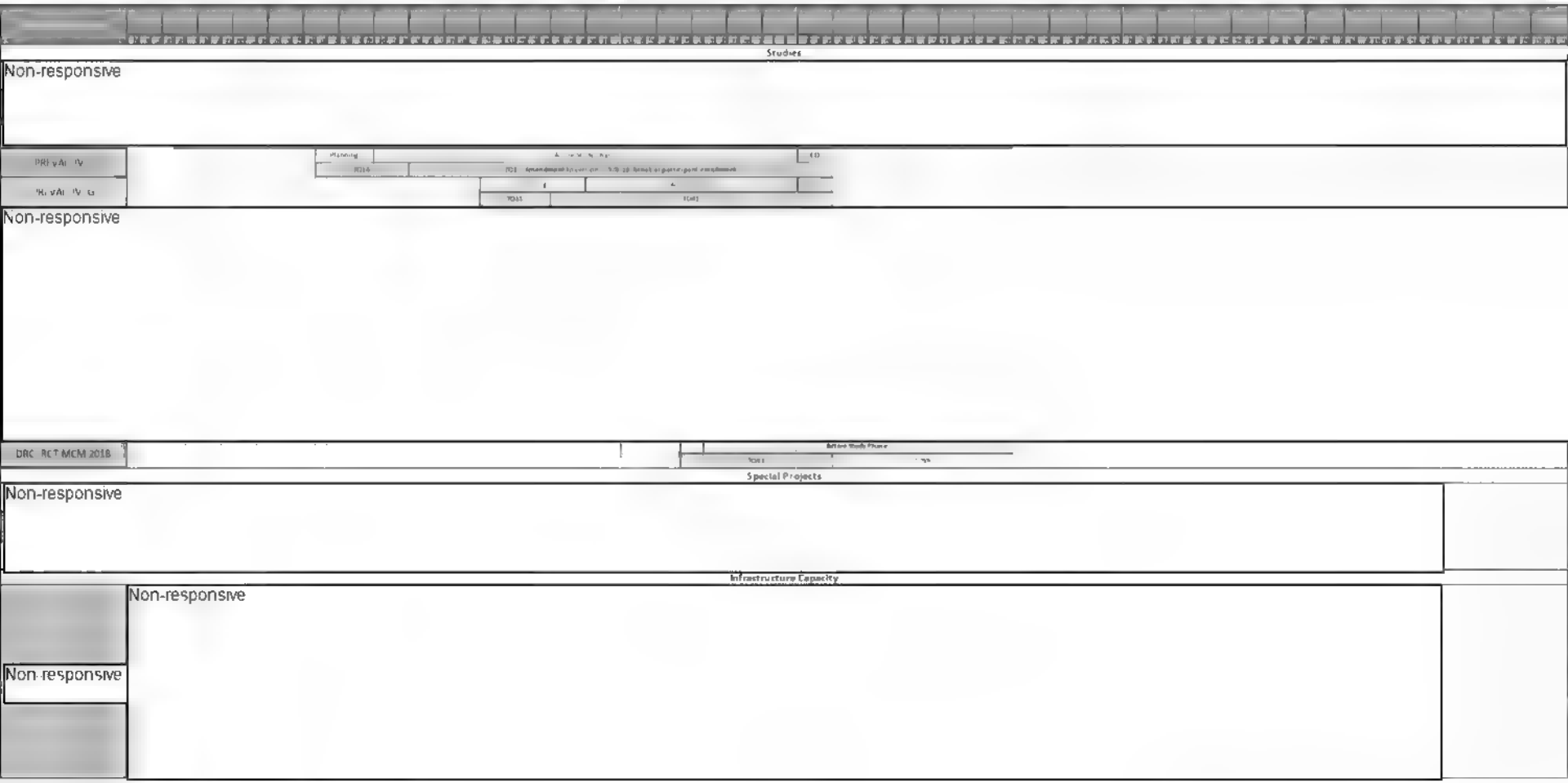
Non-responsive

October 2019 Studies Current Status

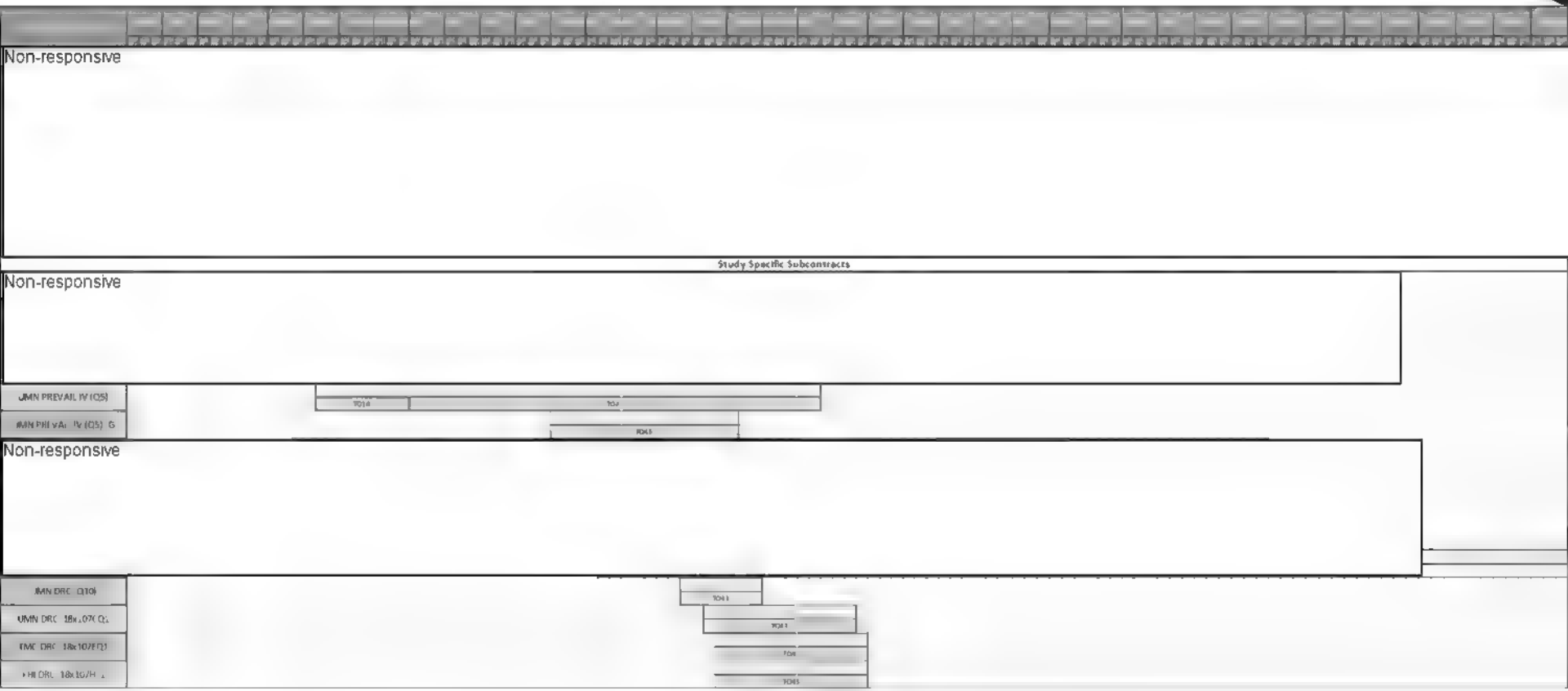
Study	Date/version	Status of recruitment	Study POP	Enrollment status	Study Site(s)
Non-responsive					
PREVAIL IV (Gilead)	May 13, 2019/v10.0	Closed to enrollment, data analysis only.	Jul 2016- Jan 2021	38/60	JFK, C.H. Rennie, Duport-Liberia, Maf - Guinea
Non-responsive					

Non-responsive

Strategy Map



Strategy Map Continued



Executive Financial Summary - Ebola: FY14-FY23

Leidos Biomed Non-Severable Funding												
Ebola Funding for West Africa & the DRC	Actual FY14	Actual FY15	Actual FY16	Actual FY17	Actual FY18	Projection FY19	Projection FY20	Projection FY21	Projection FY22	Projection FY23	Projection FY24	Total
Non-responsive												
PREVAIL IV - Liberia	-	-	425,132	730,474	421,969	105,549	-	-	-	-	-	1,683,124
PREVAIL IV - Guinea	-	-	-	93,064	1,232,437	163,152	-	-	-	-	-	1,488,653
Non-responsive												
MCM RCT in DRC	-	-	-	-	8,133	8,656,267	10,003,182	-	-	-	-	18,667,582
Non-responsive												

*No NS Funding availble past FY23

Task Order Highlights

Funding Mechanism	Status/Updates
TO 43TO 59	• Task Order 43 Non-responsive ends in FY22 and will be fully spentPoP: 9/29/2017 – 9/28/2022Funded MCM RCT in DRC through end of FY19 Non-responsive Non-responsive Non-responsive Task Order 59 Non-responsive ends in FY23PoP: 9/30/2018 – 9/29/2023 Non-responsive Non-resp Funding MCM-RCT in DRC, started 10/1/19 Non-responsive Non-responsive Non-responsive

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DCR/Leidos Biomed Financial Review of Ebola in the DRC

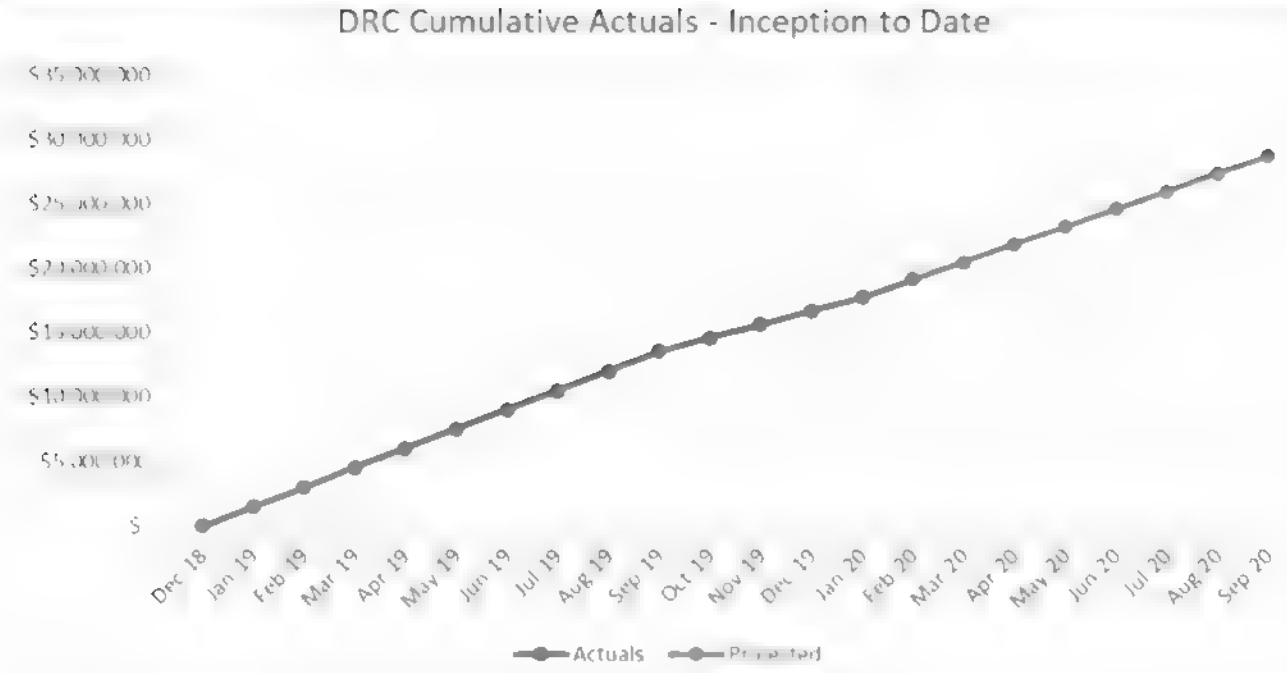
April 29, 2020: Go To Meeting Discussion

Agenda: DCR/Leidos Biomed Financial Review of Ebola in the DRC
Wednesday, April 29, 2020 1:30pm - 3:00pm

Attendees: Cliff Lane, Laura McNay, Lori Dodd, Jamila Aboulhab, Beth Grace, Rodney Ritenour, Lou Hepp, Beth Baseler, Calvin Proffitt, Kevin Newell, Matt Hohn, Ben MartinezPurpose: Review DRC financial data, including current human resources;Gain an understanding of DCR's desired contribution to this program post Ebola RCT;Initiate planning on how the desired future state might be accomplished.

Agenda:Introduction & AgendaDRC Actuals by YearLBR Task Order SummariesSubcontractor Staffing UpdatesPALM Path ForwardAdditional Discussion TopicsKey Summary Points

Summary of DRC Actuals by Year



Highlights:Total Costs:FY19:
$13,535,963 (9 months)FY20:
$15,439,687 (12 months)

Actual/Projected Monthly Burn:FY19:
$1,503,996FY20:
$1,286,641

Executive Financial Summary – All DRC Funding

Leidos Biomedical Research, Inc
Clinical Monitoring Research Program
EAC Projections / Additional Funding Requirements IDIQs TO43 & TO59

Expenses through March 27, 2020: | $ 22,027,715

Leidos Non-Severable Ebola

Base Period	FY18	FY19	FY20 EAC	FY21	FY22	FY23	Total
Non-responsive							
Milestone 2 - MCM-RCT (Existing Studies)	-	15,183	13,834,197	-	-	-	13,849,380
Non-responsive							

PALM Path Forward

Non-responsive

DRC Current vs. INRB/TMG Proposed Warm Base Staffing

Non-responsive

Scenario Planning - DRC Warm Base

Non-responsive

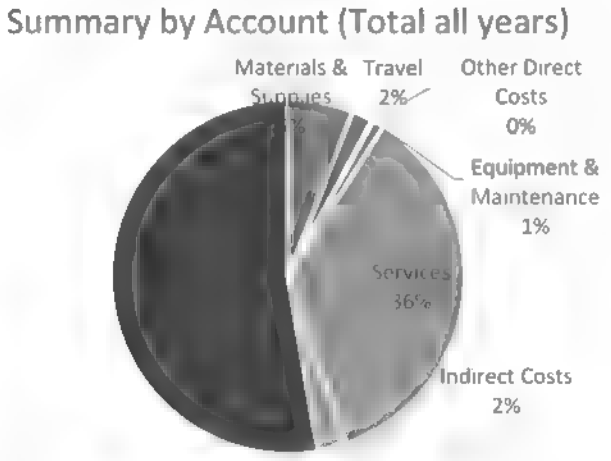
In Summary:

Non-responsive

Leidos Biomedical Research, Inc.
Clinical Monitoring Research Program
EAC Projections / Additional Funding Requirements IDIQ TO14
Leidos Non-Severable Ebola

Core Costs	FY15	FY16	FY17 EAC	FY18 EAC	FY19	FY20	FY21	Total
Non-responsive								
Incremental Cost Per Study								
Non-responsive								
PREVAIL IV - Liberia	-	569,150	3,164	-	-	-	-	572,313
PREVAIL IV - Guinea								-
Non-responsive								
Non-responsive			$ 15,960,364	Non-responsive				
Non-responsive								

Non-responsive	
Non-responsive	$ (1,597,680)
	Non-responsive



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Study Prevail IV - Libena
PID 400 075 0014 0001 585 001

	Fiscal Years	2016 EAC	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	Total	Assumptions
Direct Labor & Fringe Benefits											
	Position										
10-5311	Leidos Labor	$ 58,835	$ -	$ -	$ -	$				$ 58,835	
110	Fringe	29 712								29 712	
	Subtotal-Direct Labor & Fringe Benefits	**$ 88,547**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 88,547**	
Materials & Supplies											
15-5420-001	Occupational Clothing	$ 5 708	$ -	$ -	$ -	$ -				$ 5 708	
15-5430-001	Biologicals	97,799	576							98,375	
15-5440-001	Controlled Materials										
15-5450-001	Industrial Supplies	674								674	
15-5453-001	Tools&Test Devices										
15-5455-001	Cleaning Supplies	141								141	
15-5460-001	Lab Supplies	46 131								46,131	
15-5470-001	Office Supplies	4,159								4 159	
15-5472-001	Freight 0.52	100,304	(297)							100,008	
15-5473-001	Telephone/wireless									-	
15-5474-001	Printing & Reproduction										
15-5476-001	Dues										
15-5477-001	Books									-	
15-5480-001	Computer Hardware	5,161	-							5 161	
15-5481-001	Computer Software	4 389								4 389	
	Subtotal-Materials & Supplies	**$ 264,466**	**$ 280**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 264,746**	
Travel											
20-5511	Foreign Travel	$	$ -			$				$ -	
20-5512	Scientific Trave									-	
20-5513	Administrative Trave	22								22	
20-5514	Training										
20-5520	Travel FNL Seminars									-	
	Subtotal-Travel	**$ 22**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 22**	
Other Direct Costs											
25-5515	Recruitment	$	$	$ -	$	$				$ -	
25-5516	Relocation Expenses										
25-5519	International Health Insurance									-	
30-5331-001	Direct Labor Overtime Premium	39								39	
30-5731-001	Postage										
30-5736-001	Research Support Reimbursements										
35-5721-001	Vehicle Parts										
WH-6450-001	WH Industrial Supplies									-	
WH-6455-001	WH Cleaning Supplies		-							-	
WH-6460-001	WH Lab Supplies										
WH-6470-001	WH Office Supplies										
30-5730-001	Miscellaneous/Contingency			-						-	
	Subtotal-Other Direct Costs	**$ 39**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 39**	
Equipment & Maintenance											
40-5610	Capital Equipment	$ 34,995	$	$	$	$				$ 34,995	
40-5760-001	Service Maintenance Agreements									-	
40-5762-001	Software Support	5	-							5	
40-5763-001	Equipment Repair									-	
40-5765-001	IT Equip Maintenance										
	Subtotal-Equipment & Maintenance	**$ 35,000**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 35,000**	
Services											
50-5570-001	Consultants	$ 400	$ 2,800	$	$	$				$ 3 200	
50-5780-001	Research Support Services	146,221								146 221	
50-5790-001	Admin Support Services										
	Subtotal-Services	**$ 146,621**	**$ 2,800**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 149,421**	
Indirect Costs											
300	Materials, Equip & Subs	$ 7,981	$ 66	$	$	$	$	$	$	$ 8,046	
400	General OH	17,816								17,816	
410	A/C OH	5,727								5,727	
500	G&A	2,931	19							2 949	
	Subtotal-Indirect Costs	**$ 34,454**	**$ 84**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 34,538**	
	TOTAL ESTIMATED COST	**$ 569,150**	**$ 3,164**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ -**	**$ 572,313**	

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Leidos Biomedical Research, Inc.
Clinical Monitoring Research Program
EAC Projections / Additional Funding Requirements IDIQ TO33

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PREVAIL IV - Liberia	5,381	621,592	291,226	147,192	-	-	1,065,391
PREVAIL IV - Guinea	-	100,877	3	-	-	-	100,880

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Account Subcontracts & Consultants

PIDs ALL

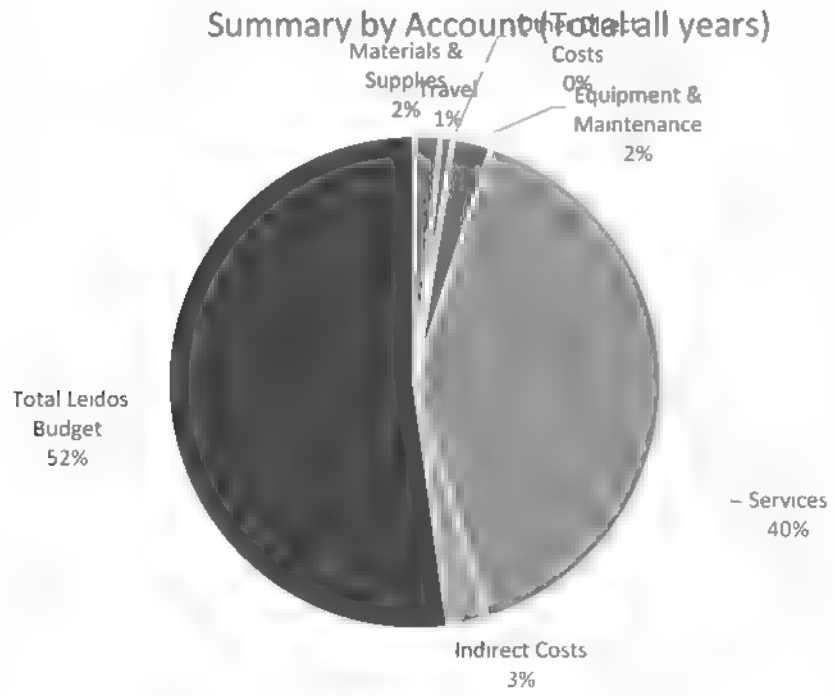
PO#:	Subcontractor	Study:	Hide Column 2016 Expenses	Hide Column 2016 ETC	2016	Hide Column 2017 Expenses	Hide Column 2017 ETC	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	Total	Assumptions
Non-responsive																
16X054Q5	The University of Minnesota	PREVAIL IV Gilead Vaccine L/G				393,372	37,060.85	430,433		228,510	228,510	114,255			773,198	
Non-responsive																
	ABMI, Inc	PREVAIL IV Gilead Vaccine G					6,385	6,385							6,385	
Non-responsive																

Allocation of Costs (Hidden)

Check = 0

Allocation of Costs (Subcontracts)	2016 Expenses	2016 TBE	2016 EAC	2017 Expenses	2017 TBE	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	Total
Non-responsive													
PREVAIL 4 Liberia				393,371.83	37.061	430,432.68		228,510	228,510	114,255			773,198
PREVAIL 4 Guinea					6.385	6,384.64							6,385
Non-responsive													

Non-responsive	
Non-responsive	$ (12,279,137)
Non-responsive	



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Study: PREVAIL IV Gilead - Liberia
PID: 400 075 0033 0001 002 585 0001

	Fiscal Years	2016 EAC	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	Total	Assumptions
Direct Labor & Fringe Benefits											
	Position										
10-5311	Leidos Labor	$	$ 49,329	$ 918	$	$ 918				$ 50,247	
110	Fringe		24,211	446		446				24,657	
	Subtotal-Direct Labor & Fringe Benefits	$ -	$ 73,540	$ 1,365	$ -	$ 1,365	$ -	$ -	$ -	$ 74,904	
Materials & Supplies											
15-5420-001	Occupational Clothing	$	$ 8,581	$	$ 4,291	$ 4,291	$ 2,145			$ 15,017	
15-5430-001	Biologicals		49,945		24,972	24,972	12,486			87,404	
15-5440-001	Controlled Materials	-	-	-	-	-	-			-	Slow enrollment
15-5450-001	Industrial Supplies		1,255		627	627	314			2,196	
15-5453-001	Tool s&Test Devices										
15-5455-001	Cleaning Supplies		144		72	72	36			251	
15-5460-001	Lab Supplies		11,492		5,746	5,746	2,873			20,111	
15-5470-001	Office Supplies		2,094		1,047	1,047	523			3,664	
15-5472-001	Freight 0 52	5,238	35,668		20,824	20,824	11,268			72,998	
15-5473-001	Telephone/wireless										
15-5474-001	Printing & Reproduction										
15-5476-001	Dues										
15-5477-001	Books										
15-5480-001	Computer Hardware		3,484							3,484	
15-5481-001	Computer Software		3,292		3,292	3,292	3,292			9,875	Sched Tool - Paid end of June
	Subtotal-Materials & Supplies	$ 5,238	$ 115,954	$ -	$ 60,871	$ 60,871	$ 32,937	$ -	$ -	$ 215,000	
Travel											
20-5511	Foreign Travel	$	$ 3,909	$ -		$				$ 3,909	
20-5512	Scientific Travel										
20-5513	Administrative Travel										
20-5514	Training										
20-5520	Travel FN, Seminars										
	Subtotal-Travel	$ -	$ 3,909	$ -	$ -	$ -	$ -	$ -	$ -	$ 3,909	
Other Direct Costs											
25-5515	Recruitment	$	$	$	$	$				$	
25-5516	Relocation Expenses										
25-5519	International Health Insurance										
30-5331-00x	Direct Labor Overtime Premium										
30-5731-00x	Postage										
30-5736-001	Research Support Reimbursements										
35-5721-001	Vehicle Parts										
WH-6450-001	WH Industrial Supplies										
WH-6455-001	WH Cleaning Supplies										
WH-6460-001	WH Lab Supplies										
WH-6470-001	WH Office Supplies										
30-5730-001	Miscellaneous/Contingency										
	Subtotal-Other Direct Costs	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	
Equipment & Maintenance											
40-5610-00x	Capital Equipment	$	$	$	$	$				$ -	
40-5760-00x	Service Maintenance Agreements										
40-5762-001	Software Support										
40-5763-001	Equipment Repair										
40-5765-001	T Equip Maintenance										
	Subtotal-Equipment & Maintenance	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$	
Services											
50-5570-001	Consultants	$	$	$	$	$				$	
50-5780-00x	Research Support Services		393,599		228,510	$ 228,510	114,255			736,364	
50-5790-001	Admin Support Services										
	Subtotal-Services	$ -	$ 393,599	$ -	$ 228,510	$ 228,510	$ 114,255	$ -	$ -	$ 736,364	
Indirect Costs											
300	Materials, Equip & Subs	$ 112	$ 11,108	$	$ 40	$ 40				$ 11,260	
400	General OH		15,331	317		317				15,648	
410	A/C OH		4,405	95		95				4,500	
500	G&A	32	3,747	15	13	28				3,806	
	Subtotal-Indirect Costs	$ 143	$ 34,591	$ 427	$ 53	$ 480	$	$ -	$ -	$ 35,214	
	TOTAL ESTIMATED COST	$ 5,381	$ 621,592	$ 1,791	$ 289,434	$ 291,226	$ 147,192	$ -	$ -	$ 1,065,391	

Study PREVAIL IV Gilead - Guinea
P.D 400 075 0033 0001 002 585 0003

	Fiscal Years	2016 EAC	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	Total	Assumptions
Direct Labor & Fringe Benefits											
	Position										
10-5311	Leidos Labor	$ -	$ 27,696	$ 0	$ -	$ 0				$ 27,696	
110	Fringe		13,593	-		-				13,593	
	Subtotal-Direct Labor & Fringe Benefits	$ -	$ 41,288	$ 0	$ -	$ 0	$ -	$ -	$ -	$ 41,289	
Materials & Supplies											
15-5420-001	Occupational Clothing	$	$	$	$	$				$	
15-5430-001	Biologicals		9,770								9,770
15-5440-00▲	Controlled Materials										
15-5450-00▲	Industrial Supplies										
15-5453-001	Tool s&Test Devices										
15-5455-001	Cleaning Supplies										
15-5460-00▲	Lab Supplies		2,000								2,000
15-5470-00▲	Office Supplies										
15-5472-00▲	Freight 0.52		470								470
15-5473-001	Telephone/wireless										
15-5474-001	Printing & Reproduction										
15-5476-001	Dues										
15-5477-00▲	Books										
15-5480-00▲	Computer Hardware										
15-5481-001	Computer Software										
	Subtotal-Materials & Supplies	$ -	$ 12,240	$ -	$ -	$ -	$ -	$ -	$ -	$ 12,240	
Travel											
20-5511	Foreign Travel	$ -	$ -	$ -	$ -	$				$ -	
20-5512	Scientific Travel										
20-5513	Administrative Travel										
20-5514	Training										
20-5520	Travel FN, Seminars										-
	Subtotal-Travel	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	
Other Direct Costs											
25-5515	Recruitment	$	$	$	$	$				$	
25-5516	Relocation Expenses										
25-5519	International Health Insurance										
30-5331-00▲	Direct Labor Overtime Premium										
30-5731-00▲	Postage										
30-5736-00▲	Research Support Reimbursements										
35-5721-001	vehicle Parts										
WH-6450-001	WH Industrial Supplies										
WH-6455-001	WH Cleaning Supplies										
WH-6460-00▲	WH Lab Supplies										
WH-6470-001	WH Office Supplies										
30-5730-001	Miscellaneous/Contingency										
	Subtotal-Other Direct Costs	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	
Equipment & Maintenance											
40-5610-00▲	Capital Equipment	$	$	$	$	$				$	
40-5760-00▲	Service Maintenance Agreements										
40-5762-001	Software Support										
40-5763-001	Equipment Repair										
40-5765-001	T Equip Maintenance										
	Subtotal-Equipment & Maintenance	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$	
Services											
50-5570-001	Consultants	$	$	$	$	$				$	
50-5780-00▲	Research Support Services		6,385								6,385
50-5790-001	Admin Support Services		28,270								28,270
	Subtotal-Services	$ -	$ 34,655	$ -	$ -	$ -	$ -	$ -	$ -	$ 34,655	
Indirect Costs											
300	Materials, Equip & Subs	$	$ 1,012	$	$	$				$ 1,012	
400	General OH		8,608								8,608
410	A/C OH		2,473								2,473
500	G&A		600	3		3					603
	Subtotal-Indirect Costs	$ -	$ 12,693	$ 3	$ -	$ 3	$ -	$ -	$ -	$ 12,696	
	TOTAL ESTIMATED COST	$ -	$ 100,877	$ 3	$ -	$ 3	$ -	$ -	$ -	$ 100,880	

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Leidos Biomedical Research, Inc.
Clinical Monitoring Research Program
EAC Projections / Additional Funding Requirements OTS, IDIQ TO14, TO33, & TO43
Leidos Non-Severable Ebola

Core Costs	FY14	FY15	FY16	FY17	FY18	FY19	FY20	FY21	Total
West Africa - Core Ops - TO33	-	-	-	11,408,780	8,881,252	7,926,640	6,490,967	4,977,472	39,685,112

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Core Costs	FY14	FY15	FY16	FY17	FY18	FY19	FY20	FY21	Total
PREVAIL IV - TO33 Liberia	-	-	5,378	691,610	591,017	279,759	-	-	*1,567,764*
PREVAIL IV - TO14 Liberia	-	-	566,064	3,162	-	-	-	-	*569,226*
Subtotal - Prev IV Liberia	$ -	$ -	$ 571,442	$ 694,772	$ 591,017	$ 279,759	$ -	$ -	$ 2,136,990

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Non-responsive	$ (32,309,910)

Leidos Biomedical Research, Inc.
Clinical Monitoring Research Program
EAC Projections / Additional Funding Requirements OTS, IDIQ TO14, TO33, & TO43

Leidos Non-Severable Ebola

Core Costs	FY14	FY15	FY16	FY17	FY18	FY19	FY20	FY21	Total
Non-responsive									
Incremental Cost Per Study									
Non-responsive									
PREVAIL IV - Liberia	-	-	571,442	694,772	591,017	279,759	-	-	2,136,990
PREVAIL V - Guinea		-	-	99,140	1,058,997	126,638	-	-	1,284,775
Non-responsive									
Non-responsive							(16,326,851)	(32,309,910)	(32,309,910)

Account Subcontracts & Consultan s

PIDs ALL

POR	Subcontractor	Study	Hide Column 2016 Expenses	Hide Column 2016 ETC	2016	Hide Column 2017 Expenses	Hide Column 2017 ETC	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	2022	Total	Assumptions
Non-responsive																	
16X054QS	The University of Minnesota	PREVAIL IV Gilead Vaccine L	71,221		71,221	426,964	33,592	460,556	100 100	414,081	514 181	248,449				1 294.407	
16X054QS	The University of Minnesota	PREVAIL IV Gilead Vaccine G								277 564	277 564	69,391				346,955	
Non-responsive																	
16X055Q	The Mitchell Group PREVAIL IV Guinea	PREVAIL IV Guinea							12,909	134,979	147,888	25,300				173,188	
Non-responsive																	
16X056Q	ABML, Inc.	PREVAIL IV Gilead Vaccine G								20,000	20,000					20,000	
Non-responsive																	
	PREVAIL 4 Liberia		143,270		143,270	476 964	33 592	460,556	100 100	414,081	514 181	248,449				1 366.456	
	PREVAIL 4 Guinea								12,909	432 543	445 452	94 691				540,143	
Non-responsive																	

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Study: Overarching Costs
P Ds

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Fiscal Years	2014	2015	2016	2017	2018 Exp	2018 ETC	Total	Assumptions
Direct Labor &								
10-53		2,823,933	64,500	34			2,888,774	
10		27 906	32 529	363			304,642	
	$	$ 3,095,839	$ 97,073	$ 503	$	$	$ 3,193,415	
Materials & Su								
15-5420-001		32 326					32 326	
5-5440-001		124 763		13 284			138,046	
5-5440-00								
5-5450-00		42,969	270				43,239	
5-5453-00								
5-5455-001		1,351					1,351	
5-5460-001		145 761		10,909			156,669	
5 5470-001		49 130	9	1 593			50 732	
5-5474-001	14	1,220,645	222	378			1 221 258	
5 5473-001		23,683	21 270	5 097			50,051	
5-5474-001								
5 5476-00				169			169	
5-5477-00			1,096				1,096	
5-5480-00		105 792	438				106,230	
15-5481-001		8 435					8,435	
	$ 14	$ 1,754,856	$ 23,305	$ 31,429			$ 1,809,604	
Travel								
20-55		867 639	234 205	104 983	72 999		1 279,826	
20-5512								
20-55		1 275	(2 266)				(991	
20-55		12 001	130				12 131	
20-5520			(2 572				(2,572	
	$	$ 880,915	$ 229,497	$ 104,983	$ 72,999	$	$ 1,288,394	
Other Direct Co								
25-55								
25-5516								
25-5519		17 198	2 926				20 124	
30-5331								
30-5731		14,392	1 965				16,357	
30-5736								
35-5721								
WH-6450-001								
WH-6455-001								
WH-6460-00								
WH-6470-001								
30-5730-001		266 723	224				266 947	
	$	$ 298,313	$ 5,115	$	$	$	$ 303,427	
Equipment & M								
40-56 0		565 974					565 974	
40-5760-001		2 339	8 032				10,371	
40-576 -001								
40-5763-001								
40-5765-001		1 733					1 733	
	$	$ 570,046	$ 8,032	$	$	$	$ 578,078	
Services								
50-5970-001		201 563	3 025				204 588	
50-5780-001		9 433 483	3,021 976				12 455 459	
50-5785-001								
50-5790-001		28 948					28 948	
	$	$ 9,663 993	$ 3,025,001	$	$	$	$ 12,688,994	
Indirect Costs								
300		63 540	64 152	659			128,351	
400			19 531	105			19 636	
410			6 279	30			6 308	
500		18 317	20 170	869	620		40 177	
	$	$ 81,858	$ 110,331	$ 1,663	$ 620	$	$ 194,472	
	$ 14	$ 16,350,819	$ 3,493,354	$ 138,578	$ 73,620	$	$ 20,056,386	

	FY18	FY19	FY20
	48 57%	49 38%	49.29%
	2 80%	3 51%	3 59%
	34 47%	28 21%	27 94%
CRD	10 37%	10 06%	10 04%
	0 85%	1 05%	1 06%

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Study PRE vAIL IV Gilead Liberia
P D 400 075 0033 0001 002 585 0001

Fiscal Years	Hide Column 2016 Expenses	Hide Column 2016 ETC	2016 EAC	Hide Column 2017 Expenses	Hide Column 2017 ETC	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	Total	Assumptions
Direct Labor & Fringe Benefits														
Position														
10-5311 Leidos Labor	58,835		58,835	49,329		49,329	12,299		12,299				120,463	
10 Fringe	29,712		29,712	23,523		23,523	5,974		5,974				59,209	
Subtotal-Direct Labor & Fringe Benefits	$ 88,547	$	$ 88,547	$ 72,852	$	$ 72,852	$ 18,273	$	$ 18,273	$	$	$	$ 179,673	
Materials & Supplies														
15-5420-001 Occupational Clothing	5,708		5,708	7,355	1,226	8,581		2,145	2,145	1,073			17,507	
5-5430-001 Biologicals	97,799		97,799	50,521	269	50,791	17,712	(5,158)	12,554	6,277			167,420	
25-5440-001 Controlled Materials														
25-5450-001 Industrial Supplies	674		674	1,255		1,255	700	970	1,670	835			4,434	
25-5453-001 Tools&Test Devices														
25-5455-001 Cleaning Supplies	141		141	144		144		36	36	18			338	
25-5460-001 Lab supplies	46,831		46,831	11,492		11,492	1,561	1,362	2,923	1,437			61,933	
25-5470-001 Office Supplies	4,459		4,459	2,094		2,094	1,492	523	2,016	1,008			9,576	
25-5472-001 Freight	105,542		105,542	36,867		36,867	4,804	1,648	6,452	7,248			156,110	
25-5473-001 Telephone/wireless														
25-5474-001 Printing & Reproduction														
25-5475-001 Dues														
25-5477-001 Books														
25-5480-001 Computer Hardware	5,161		5,161	3,484		3,484	49		49				8,695	
25-5481-001 Computer Software	4,389		4,389	3,292		3,292		3,292	3,292				4,264	Appt Plus Pd June annually
Subtotal-Materials & Supplies	$ 269,704	$	$ 269,704	$ 116,504	$ 1,495	$ 117,999	$ 26,268	$ 6,819	$ 31,087	$ 21,187	$	$	$ 439,977	
Travel														
20-5511 Foreign Travel				3,909	-	3,909	1,712		1,712				5,621	
20-55 2 Scientific Travel														
20-553 Administrative Travel	22												22	
20-554 Training														
20-5520 Travel-FNL Seminars														
Subtotal Travel	$ 22	$ -	$ 22	$ 3,909	$ -	$ 3,909	$ 1,712	$	$ 1,712	$	$	$	$ 5,643	
Other Direct Costs														
25-5535 Recruitment														
25-5536 Relocation Expenses														
25-5539 International Health Insurance														
30-5331-001 Direct Labor Overtime Premium	39		39										39	
30-5731-001 Postage														
30-5[illegible]10 Research Support Reimbursements														
35-5721-001 Vehicle Parts														
WH-6450-00 WH Industrial Supplies														
WH-6455-00 WH Cleaning Supplies														
WH-6460-001 WH Lab Supplie														
WH-6470-00 WH Office Supplies														
30-5730-00 Miscellaneous/Contingency														
Subtotal-Other Direct Costs	$ 39	$	$ 39	$	$	$	$	$	$	$	$	$	$ 39	
Equipment & Maintenance														
40-5[illegible] Capital Equipment	34,995		34,995										34,995	
40-5760-001 Service Maintenance Agreements														
40-5764-00 Software Suppor	5		5										5	
40-5765-00 Equipment Repair														
40-5765-001 Equip Maintenance														
Subtotal-Equipment & Maintenance	$ 35,000	$	$ 35,000	$	$	$	$	$	$	$	$	$	$ 35,000	
Services														
50-5[illegible] Consultants	400		400	2,800		2,800							3,200	
50-5780-00 Research Support Services	143,270		143,270	426,964	33,592	460,556	100,100	424,081	524,181	248,449			1,166,456	
50-5785-001 Services Commercial														
50-5790-00 Admin Support Services														
Subtotal-Services	$ 143,670	$	$ 143,670	$ 429,764	$ 33,592	$ 463,356	$ 100,100	$ 424,081	$ 524,181	$ 248,449	$	$	$ 1,169,656	
Indirect Costs														
300 Materials Equip & Subs	7,979		7,979	11,860	982	12,843	3,538	11,729	15,267	7,739			43,828	
400 General OH	17,816		17,816	15,167		15,167	8,240		8,240				37,222	
410 A/C OH	5,727		5,727	4,341		4,341	1,225		1,225				11,324	
500 G&A	2,939		2,939	4,019	307	4,326	1,372	3,660	4,981	2,385			14,631	
Subtotal-Indirect Costs	$ 34,460	$	$ 34,460	$ 35,367	$ 1,289	$ 36,656	$ 10,374	$ 15,390	$ 25,764	$ 10,124	$	$	$ 107,004	
TOTAL ESTIMATED COST	$ 571,442	$ -	$ 571,442	$ 668,395	$ 36,376	$ 804,772	$ 156,727	$ 434,289	$ 591,017	$ 279,759	$	$	$ 2,136,990	

Overhead Rates	FY 6	FY 7	FY17	FY17				FY18	FY19	FY20	FY21
Fringe *applied to Direct Labor*	50 50%	47 69%	47 69%	47 69%				48 57%	49 38%	49 29%	49 29%
MES *applied to Total MES*	2 13%	2 08%	2 08%	2 08%				2 80%	3 51%	3 59%	3 59%
General OH *applied to Direct Labor*	30 28%	30 75%	30 75%	30 75%				34 47%	28 21%	27 94%	27 94%
Applied/Clinica OH *applied to Direct Labor supported by ADRD or*	9 73%	8 76%	8 76%	8 76%				10 37%	10 06%	10 04%	10 04%
G&A *applied Total Direct Costs + Fringe + M&S + All OH*	0 59%	0 58%	0 58%	0 58%				0 85%	1 05%	1 06%	1 06%

Study PREVAIL IV Gilead - Guinea
PID 400.075.0033.0001 002 585 0003

Fiscal Years	2016 Expenses	2016 ETC	2016 EAC	2017 Expenses	2017 ETC	2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	Total	Assumptions
Direct Labor & Fringe Benefits														
Position														
10-5311 Leidos Labor				27,696		27,696	0		0				27,696	
510 Fringe				13,207		13,207							13,207	
Subtotal-Direct Labor & Fringe Benefits	$	$	$	$ 40,903	$	$ 40,903	$ 0	$	$ 0	$	$	$	$ 40,903	
Materials & Supplies														
15 5420-001 Occupational Clothing														
5 5430-001 Biologicals				9,770		9,770							9,770	
5 5440-001 Controlled Materials														
5 5450-001 Industrial Supplies														
5 5453-001 Tools&Test Devices														
5 5455-001 Cleaning Supplies														
5 5460-001 Lab Supplies				2,000		2,000	124		124				2,124	
5 5470-001 Office Supplies														
5 5472-001 Freight	0.52			470		470	5,106		5,106				5,576	
5 5473-001 Telephone/wireless														
5 5474-001 Printing & Reproduction														
5 5476-001 Dues														
5 5477-001 Books														
5 5480-001 Computer Hardware														
5 5481-001 Computer Software														
Subtotal-Materials & Supplies	$	$	$	$ 12,240	$	$ 12,240	$ 5,230	$	$ 5,230	$	$	$	$ 17,470	
Travel														
20-5511 Foreign Travel							640		640				640	
20-55 2 Scientific Travel														
20 553 Administrative Travel														
20-55 4 Training														
20-5520 Trave: FNL Seminars														
Subtotal Travel	$	$	$	$	$	$	$ 640	$	$ 640	$	$	$	$ 640	
Other Direct Costs														
25 5535 Recruitment														
25 5536 Relocation Expenses														
25 55 9 International Health Insurance														
30 5 3 00 Direct Labor Overtime Premium														
30 57 00 Postage														
30 5 5 00 Research Support Reimbursements														
35 57 00 Vehicle Par														
WH 6450 01 WH Industrial Supplies														
WH 6455 01 WH Cleaning Supplies														
WH 6460 01 WH Lab Supplie														
WH 6470 01 WH Office Supplies														
10 5730 00 Miscellaneous/Contingency														
Subtotal-Other Direct Costs	$	$	$	$	$	$	$	$	$	$	$	$	$	
Equipment & Maintenance														
40 5610 Capital Equipment														
40-5760-001 Service Maintenance Agreements														
40 5764 00 Software Suppor														
40-5765 00 Equipment Repair														
40-5765-001 Equip Maintenance														
Subtotal-Equipment & Maintenance	$	$	$	$	$	$	$	$	$	$	$	$	$	
Services														
50 5710 00 Consultants														entered in 5780-s/b 5790
50-5780-00 Research Support Services														
50-5785-001 Services Commercial														
50 5790 00 Admin Support Services				33,452		33,452							33,452	
Subtotal-Services	$	$	$	$ 33,452	$	$ 33,452	$	$	$	$	$	$	$ 33,452	
Indirect Costs														
300 Materials Equip & Subs				991		991	146		146				1,137	
400 General OH				8,515		8,515							8,515	
410 A/C OH				2,426		2,426							2,426	
500 G&A				592		592	51		51				643	
Subtotal-Indirect Costs	$	$	$	$ 12,524	$	$ 12,524	$ 196	$	$ 196	$	$	$	$ 12,721	
TOTAL ESTIMATED COST	$	$	$	$ 99,119	$	$ 99,119	$ 6,067	$	$ 6,067	$	$	$	$ 105,196	

Overhead Rates	FY 6	FY 7	FY17	FY17			FY18	FY19	FY20	FY21
Fringe *applied to Direct Labor*	50 50%	47 69%	47 69%	47 69%			48 57%	49 38%	49 29%	49 29%
MES *applied to Total MES*	2 13%	2 08%	2 08%	2 08%			[illegible]	3 51%	3 59%	3 59%
General OH *applied to Direct Labor*	30 28%	30 75%	30 75%	30 75%			34 47%	28 21%	27 94%	27 94%
Applied/Clinica OH *applied to Direct Labor supported by ADRD or*	9 73%	8 76%	8 76%	8 76%			10 37%	10 06%	10 04%	10 04%
G&A *applied Total Direct Costs + Fringe + M&S + All OH*	0 59%	0 58%	0 58%	0 58%			0 85%	1 05%	1 06%	1 06%

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Study: PREVAIL IV Gilead - Guinea
PID: 400.075.0043.0001.001.585.0003

Fiscal Years:			2017 EAC	2018 Expenses	2018 ETC	2018 EAC	2019	2020	2021	2022	Total	Assumptions
Direct Labor & Fringe Benefits												
	Position											
10-5311	Leidos Labor		-	104,757	-	104,757	-	-	-	-	104,757	
110	Fringe		-	50,880	-	50,880	-	-	-	-	50,880	
	Subtotal-Direct Labor & Fringe Benefits		$ -	$ 155,637	$ -	$ 155,637	$ -	$ -	$ -	$ -	$ 155,637	
Materials & Supplies												
15-5420-001	Occupational Clothing		-	10,603	2,464	13,067	2,000	-	-	-	15,067	
15-5430-001	Biologicals		-	109,968	25,490	135,458	10,503	-	-	-	145,961	
15-5440-001	Controlled Materials		-	-	-	-	-	-	-	-	-	
15-5450-001	Industrial Supplies		-	20,679	45	20,725	-	-	-	-	20,725	
15-5453-001	Tools&Test Devices		-	-	-	-	-	-	-	-	-	
15-5455-001	Cleaning Supplies		-	3,015	-	3,015	-	-	-	-	3,015	
15-5460-001	Lab Supplies		-	35,195	12,390	47,585	5,000	-	-	-	52,585	
15-5470-001	Office Supplies		-	14,616	70	14,686	500	-	-	-	15,186	
15-5472-001	Freight	0.52	21	49,736	22,457	72,193	9,362	-	-	-	81,554	
15-5473-001	Telephone/wireless		-	590	-	590	-	-	-	-	590	
15-5474-001	Printing & Reproduction		-	-	-	-	-	-	-	-	-	
15-5476-001	Dues		-	-	-	-	-	-	-	-	-	
15-5477-001	Books		-	-	-	-	-	-	-	-	-	
15-5480-001	Computer Hardware		-	1,856	-	1,856	-	-	-	-	1,856	
15-5481-001	Computer Software		-	1,661	2,728	4,389	-	-	-	-	4,389	Appt Plus-annually
	Subtotal-Materials & Supplies		$ 21	$ 247,918	$ 65,644	$ 313,563	$ 27,364	$ -	$ -	$ -	$ 340,927	
Travel												
20-5511	Foreign Travel		-	-	-	-	-	-	-	-	-	
20-5512	Scientific Travel		-	-	-	-	-	-	-	-	-	
20-5513	Administrative Travel		-	-	-	-	-	-	-	-	-	
20-5514	Training		-	-	-	-	-	-	-	-	-	
20-5520	Travel FNL Seminars		-	-	-	-	-	-	-	-	-	
	Subtotal-Travel		$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	
Other Direct Costs												
25-5515	Recruitment		-	-	-	-	-	-	-	-	-	
25-5516	Relocation Expenses		-	-	-	-	-	-	-	-	-	
25-5519	International Health Insurance		-	-	-	-	-	-	-	-	-	
30-5331-001	Direct Labor Overtime Premium		-	-	-	-	-	-	-	-	-	
30-5731-001	Postage		-	-	-	-	-	-	-	-	-	
30-5736-001	Research Support Reimbursements		-	-	-	-	-	-	-	-	-	
35-5721-001	Vehicle Parts		-	-	-	-	-	-	-	-	-	
WH-6450-001	WH Industrial Supplies		-	-	-	-	-	-	-	-	-	
WH-6455-001	WH Cleaning Supplies		-	-	-	-	-	-	-	-	-	
WH-6460-001	WH Lab Supplies		-	-	-	-	-	-	-	-	-	
WH-6470-001	WH Office Supplies		-	-	-	-	-	-	-	-	-	
30-5730-001	Miscellaneous/Contingency		-	-	-	-	-	-	-	-	-	
	Subtotal-Other Direct Costs		$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -	$ -
Equipment & Maintenance												
40-5610	Capital Equipment		-	5,430	-	5,430	-	-	-	-	5,430	
40-5760-001	Service Maintenance Agreements		-	-	-	-	-	-	-	-	-	
40-5762-001	Software Support		-	-	-	-	-	-	-	-	-	
40-5763-001	Equipment Repair		-	-	-	-	-	-	-	-	-	
40-5765-001	IT Equip Maintenance		-	-	-	-	-	-	-	-	-	
	Subtotal-Equipment & Maintenance		$ -	$ 5,430	$ -	$ 5,430	$ -	$ -	$ -	$ -	$ 5,430	
Services												
50-5570-001	Consultants		-	-	-	-	-	-	-	-	-	
50-5780-001	Research Support Services		-	51,927	432,543	484,470	94,691	-	-	-	579,161	Alima, ABML, & UMN
50-5785-001	Services - Commercial		-	-	-	-	-	-	-	-	-	Alima, ABML, & UMN
50-5790-001	Admin Support Services		-	-	15,000	15,000	-	-	-	-	15,000	
	Subtotal-Services		$ -	$ 51,927	$ 447,543	$ 499,470	$ 94,691	$ -	$ -	$ -	$ 594,161	
Indirect Costs												
300	Materials, Equip & Subs		1	8,548	14,369	22,917	3,503	-	-	-	26,420	
400	General OH		-	36,110	-	36,110	-	-	-	-	36,110	
410	A/C OH		-	10,863	-	10,863	-	-	-	-	10,863	
500	G&A		0	4,456	4,484	8,940	1,080	-	-	-	10,020	
	Subtotal-Indirect Costs		1	$ 59,977	$ 18,853	$ 78,830	$ 4,583	$ -	$ -	$ -	$ 83,413	
	TOTAL ESTIMATED COST		$ 21	$ 520,888	$ 532,041	$ 1,052,930	$ 126,638	$ -	$ -	$ -	$ 1,179,568	